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(54) Title: SYNTHONS FOR OLIGONUCLEOTIDE SYNTHESIS

(57) Abstract: The present invention relates to phosphoramidite compounds, especially to a trivalent phosphorus multimer, a method of utilizing a trivalent phosphorus multimer to prepare an oligonucleotide, and a method of preparing a trivalent phosphorus multimer. In addition, the invention relates to a solid support that is derivatized with a trivalent phosphorus multimer and a method of preparing the same.

SYNTHONS FOR OLIGONUCLEOTIDE SYNTHESIS

BACKGROUND OF THE INVENTION

Synthetic oligonucleotides are important in the diagnostic field for the detection of genetic and viral diseases. In addition, large scale synthesis of oligonucleotides for use in antisense and related therapies has become increasingly important since FDA approval of an oligonucleotide analog for the treatment of cytomegalovirus (CMV), and several other oligonucleotide analogs are currently in clinical trials. Kilogram quantities of a purified oligonucleotide analog are needed for each clinical trial.

Oligonucleotide analogs, which contain modified linkages between nucleotides, such as phosphorothioate linkages or a combination of phosphorothioate and phosphate diester linkages (chimeric oligonucleotides), have been found to be more useful than oligonucleotides which have only phosphate diester linkages in antisense applications because they are more resistant to degradation in the body. Typically, oligonucleotide analogs are synthesized by adding each nucleotide monomer one at a time until the desired sequence is complete. Addition of each nucleotide requires a cycle which minimally has three reaction steps: a coupling step, an oxidation or sulfurization step, and a 5'-deprotection step. Typically, a capping step is also performed after the oxidation or sulfurization step. Therefore, synthesis of an oligonucleotide having 21 bases requires 20 cycles or from 60-80 reactions (depending on whether the capping step is preformed in each cycle). Since the yield in each reaction step is generally less than 100%, it is difficult to synthesize long oligonucleotides in good yield and very difficult, if not impossible, to synthesize oligonucleotides that have greater than about 150 nucleotide bases with the current technology. In addition, the large number of reaction steps uses up large quantities of reagents, thus making the synthesis more expensive. In order to obtain longer oligonucleotides in good yield and/or to reduce the cost of reagents used to synthesize oligonucleotides, it would be desirable to reduce the number of reaction steps necessary to synthesize oligonucleotides.

The use of dimers or trimers has been suggested to increase the yield and availability of longer synthetic oligonucleotides. However, dimer and trimers utilized to date have phosphorus linkages which have been either oxidized to form a phosphate diester or sulfurized to form a phosphorothioate. Therefore, these building blocks are not as versatile as monomer building blocks in which the same monomer building blocks can be used to form oligonucleotides with either a phosphate diester, a phosphorothioate backbone or a combination of phosphodiester and phosphorothioate backbone.

After synthesis an oligonucleotide is usually separated from impurities generated during synthesis. Typically, these impurities consist of reagents, reaction by-product and failure sequences. Failure sequences are oligonucleotides where one or more coupling step failed so that the failure sequence is shorter than the desired length by one or more

nucleotides. Failure sequences are generally removed by ion-exchange chromatography. However, failure sequences which are only one nucleotide shorter than the desired length (i.e. N-1 species) are difficult to remove because their structural similarity to the desired product causes them to have a similar chromatographic mobility as the product oligonucleotide. N-2 and N-3 failure sequences are more readily separated from the desired product because they differ enough in structure to cause them to have substantially different chromatographic mobilities from the product oligonucleotide. Therefore, a synthetic method that does not produce N-1 failure sequences is desirable in order to improve the yield and purity of the desired oligonucleotide.

Improvements in the synthesis of oligonucleotide and oligonucleotide analogs are necessary in order to produce these compounds in the quantity and quality necessary for therapeutic use. In addition, the ability to synthesize longer oligonucleotides in useful yields could extend their use in therapeutic and recombinant applications.

SUMMARY OF THE INVENTION

The present invention relates to a trivalent phosphorus multimer, a method of utilizing a trivalent phosphorus multimer to prepare an oligonucleotide, and a method of preparing a trivalent phosphorus multimer. In addition, the invention relates to a solid support that is derivatized with a trivalent phosphorus multimer and a method of preparing the same.

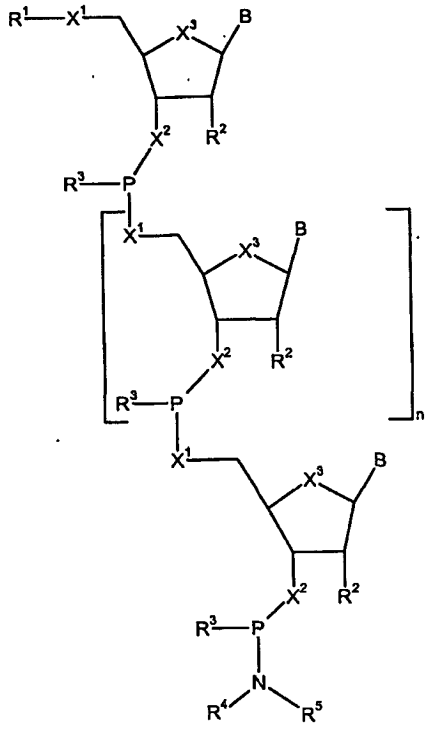
In one aspect of the present invention, there is provided a phosphoramidite compound comprising two or more nucleoside moieties linked by one or more internucleoside phosphorus atoms, wherein the internucleoside phosphorus atoms are phosphorus (III) atoms.

The phosphoramidite compounds comprise a phosphoramidite moiety which may be bonded to the 5'-position or preferably the 3'-position of the nucleoside moiety carrying the phosphoramidite moiety. The phosphoramidite moiety is preferably a group of formula $-X^1-PR^3NR^4R^5$ wherein R^3, R^4 and R^5 are as described below.

The nucleoside moieties may be d- or l-nucleosides, but in many embodiments are d-nucleosides. The nucleosides may be abasic, but in many embodiments comprises nucleobases. Commonly, the nucleobases are protected by suitable protecting groups known in the art.

The internucleoside phosphorus (III) atom preferably comprises a phosphite triester group. In many embodiments, the phosphite triester group links the 5'-position of the nucleoside moiety carrying the phosphoramidite moiety with the 3'-position of a second nucleoside moiety. Most preferably, the phosphite triester group comprises a beta-cyanoethyloxy or beta-cyanoethylthio moiety.

In another aspect, there is provided a trivalent phosphorus multimer which can be represented by Structural Formula I, or a stereoisomer thereof:

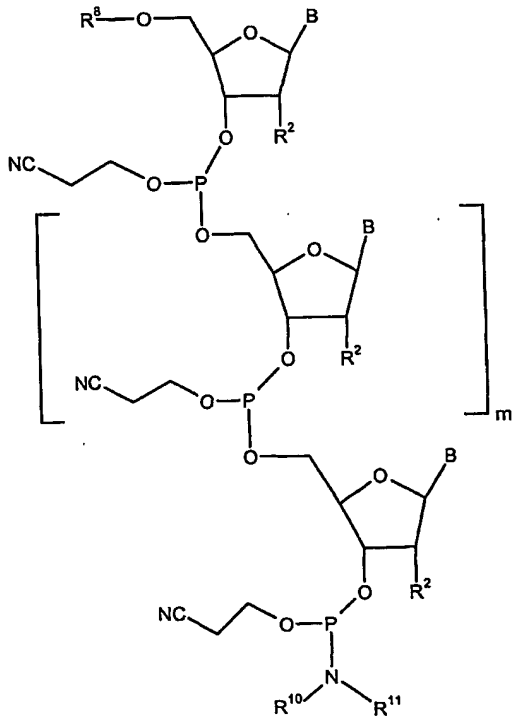


I.

In Structural Formula I, each X^1 is, independently, -O- or -S-. Each X^2 is, independently, -O-, -S-, or -NR-. Each X^3 is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$. In a preferred embodiment, each X^1, X^2, and X^3 is -O-. R^1 is a protecting group, preferably an acid labile protecting group or a trialkylsilyl group, such as *t*-butyldimethylsilyl or triisopropylsilyl. In a more preferred embodiment, R^1 is a substituted or unsubstituted trityl, 9-(phenyl-) xanthenyl (hereinafter "pixyl") or tetrahydropyranyl (hereinafter "THP"). In an even more preferred embodiment, R^1 is an unsubstituted trityl, a monoalkoxytrityl, a dialkoxytrityl, a trialkoxytrityl, THP or pixyl. Most preferably, R^1 is 4,4'-dimethoxytrityl. Each R^2 is, independently, -H, -F, $-NHR^6$, $-CH_2R^6$ or $-OR^6$. Each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group or a group of formula $-OR^7$ or $-SR^7$. Preferably, each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, 4-cyanobut-2-enylthio, 4-cyanobut-2-enyloxy, allylthio, allyloxy, crotylthio, or crotyloxy. R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl group; or R^4 and R^5 taken together with the nitrogen to which they are bonded form a heterocycloalkyl group or a heteroaromatic group, in which the heterocycloalkyl or heteroaromatic ring is preferably a five or six membered ring. Preferably, each R^4 and R^5 is an isopropyl group. R is -H, a substituted or

unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, or an amine protecting group. R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or a protecting group, such as an alcohol protecting group, for example *t*-butyldimethylsilyl, or an amine protecting group. R^7 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl group, THP, 4-methoxytetrahydropyranyl or 2-fluorophenyl-methoxypiperidin-4-yl. In a specific embodiment, R^7 is *o*-chlorophenyl or *p*-chlorophenyl. Each B is, independently, a protected or an unprotected nucleoside base or may represent H when one or more abasic moieties are present. n is 0 or a positive integer. When a trivalent phosphorus multimer of the invention is used to synthesize an oligonucleotide on a solid support, n is preferably 0 to 2. Preferably, n is 0 to 10 when a trivalent phosphorous multimer is used to synthesize an oligonucleotide in solution. In certain embodiments, R^2 represents a C-allyl group. Most preferably, R^2 represents H, O or OCH_2CH_2OMe.

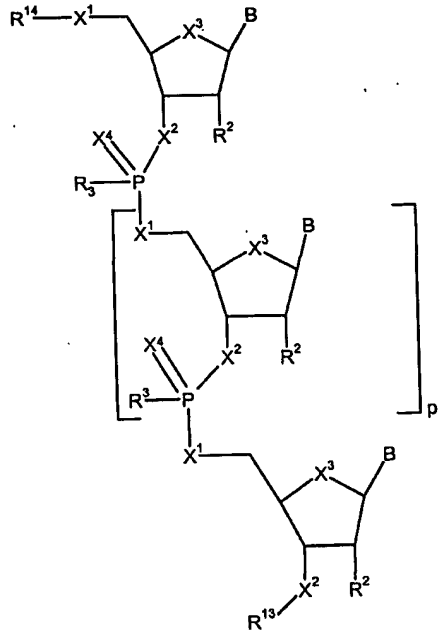
In one embodiment, the trivalent phosphorus multimer can be represented by structural Formula II, or a stereoisomer thereof:



II.

In Structural Formula II, B and R^2 are defined as for Structural Formula I. R^8 is a substituted or unsubstituted trityl, such as 4,4'-dimethoxytrityl. R^{10} and R^{11} are each, independently a substituted or unsubstituted aliphatic group. Preferably, each R^{10} and R^{11} is an isopropyl group. m is 0 or 1.

The trivalent phosphorus multimer represented by Structural Formula I can be used to prepare an oligonucleotide represented by Structural Formula III, or a stereoisomer thereof:

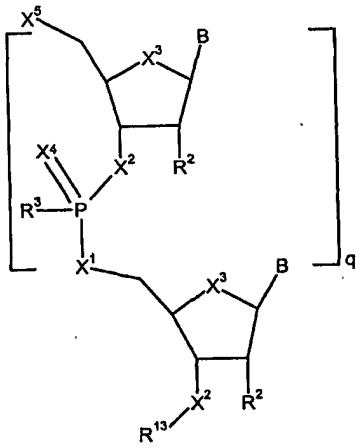


III.

In Structural Formula III, X^1, X^2, X^3, R^2, R^3 and B are as defined for Structural Formula I. Each X^4 is, independently, =O or =S. R^{13} is a hydroxyl protecting group, a thiol protecting group, an amine protecting group, a group of the formula $-Y^2-L-Y^1$, a solid support or a group of the formula $-Y^2-L-Y^2-R^{15}$. Y^1 is a functional group which can react with an amine, thiol or hydroxyl group. Preferably, Y^1 is an ester or a carboxylic acid group. Each Y^2, is, independently, a single bond, -C(O)-, $-C(O)NR^{17}-$, $-C(O)O-$, $-NR^{17}-$ or -O-. L is a linker which is preferably a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group. More preferably, L is a ethylene group. R^{17} is -H, a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group. R^{15} is a solid support suitable for solid phase oligonucleotide synthesis, such as controlled-pore glass, polystyrene, microporous polyamide such as polydimethylacrylamide, polystyrene coated with polyethylene glycol, and polyethylene

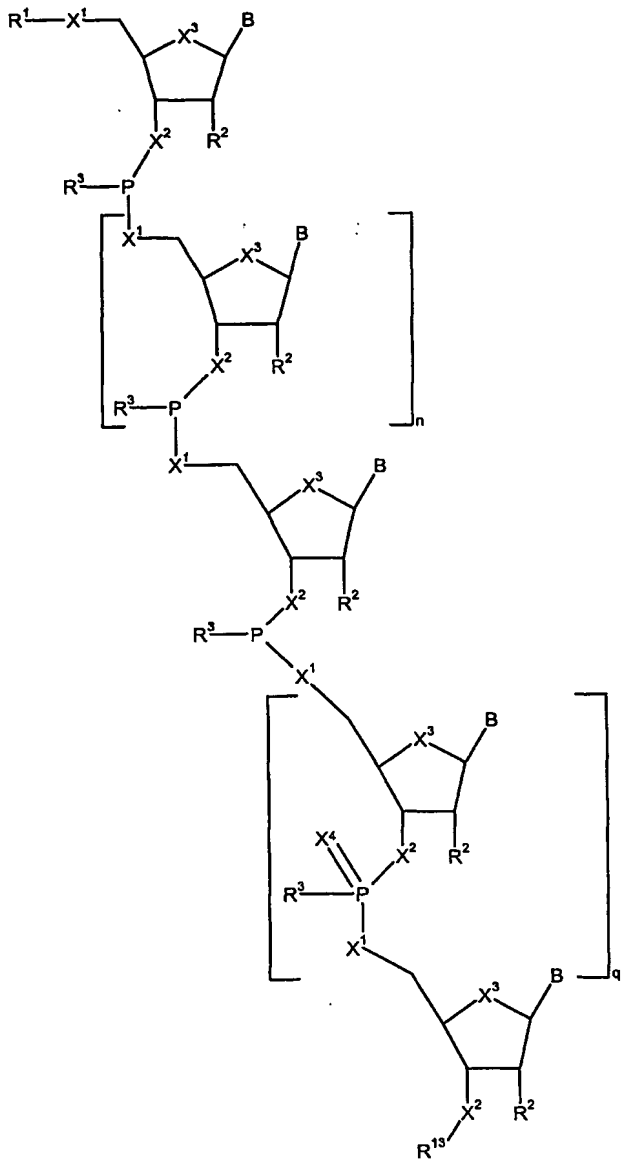
glycol supported on polystyrene, such as those solid supports commercially available under the trade name Tentagel. R^{14} is -H or a protecting group. Preferably, when R^{14} is a protecting group, it is an acid labile protecting group, *t*-butyldimethylsilyl or triisopropylsilyl. p is a positive integer. In one embodiment, the oligonucleotide synthesized with the trivalent phosphorus multimers is a phosphate and, therefore, has only phosphate linkages (ie each internucleotide phosphorus is bonded only to oxygen). In another embodiment, the oligonucleotide synthesized is a phosphorothioate and, therefore, has only phosphorothioate linkages (each internucleotide phosphorus is bonded to at least one S, preferably only one S). In yet another embodiment, the oligonucleotide synthesized is a chimeric oligonucleotide which comprises both phosphate and phosphorothioate internucleotide linkages.

In the method of preparing the oligonucleotide represented by Structural Formula III, a trivalent phosphorus multimer represented by Structural Formula I is coupled to a 5'-deprotected nucleotide or nucleoside represented by Structural Formula IV, or a stereoisomer thereof:



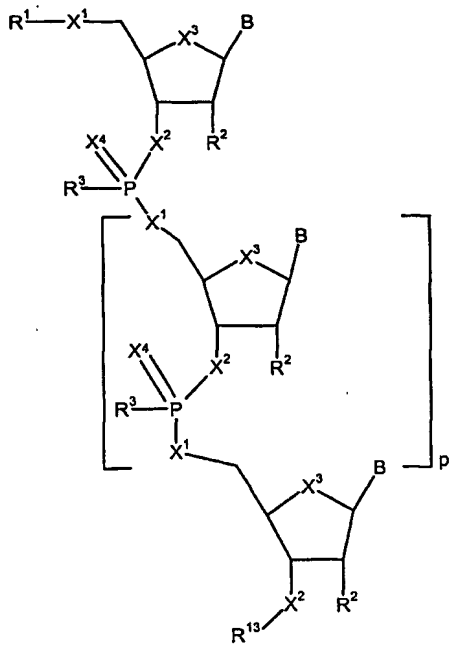
IV.

In Structural Formula IV, X^1, X^2, X^3, X^4, R^2, R^3, R^{13}, and B are defined as above. X^5 is -OH or -SH. q is 0 or a positive integer. The coupling reaction forms a first intermediate represented by Structural Formula V, or a stereoisomer thereof:



V.

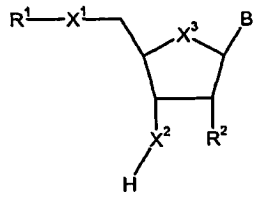
In Structural Formula V, X^1, X^2, X^3, X^4, R^1, R^2, R^3, R^{13}, B, n and q are defined as above. The trivalent phosphorus groups of the first intermediate are then oxidized or sulfurized to form a second intermediate represented by Structural Formula VI, or a stereoisomer thereof:



VI.

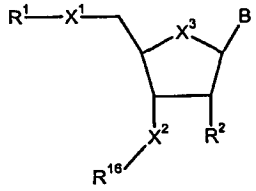
In Structural Formula VI, R^1, R^2, R^3, R^{13}, X^1, X^2, X^3, X^4, B and p are as defined above. Any 5'-deprotected nucleoside or nucleotide represented by Structural Formula IV which remains in the reaction mixture can optionally be treated with a reagent to cap the unreacted X^5 groups. The second intermediate is then treated to remove R^1. If R^1 is an acid labile protecting group, the second intermediate is treated with an acid to remove R^1. If R^1 is a trialkylsilyl group, such as *t*-butyldimethylsilyl group or a triisopropylsilyl group, the second intermediate can be treated with fluoride ions to remove R^1. Typically, *t*-butyldimethylsilyl and a triisopropylsilyl are removed by treatment with a solution of tetrabutylammonium fluoride in THF. Methods for removing *t*-butyldimethylsilyl can be found in Greene, *et al.*, *Protective Groups in Organic Synthesis* (1991), John Wiley & Sons, Inc., pages 77-83, the teachings of which are incorporated herein by reference in their entirety. The above reaction steps, or reaction cycle, can be repeated one or more times to form an oligonucleotide of the desired length. When it is desired to obtain an oligonucleotide product in which the 5'-end group is protected, the final step of the reaction cycle can be the capping step, if a capping step is done, or the final step of the reaction can be an oxidation or sulfurization step if a capping step is not done. Alternatively, the final step of the reaction cycle can be removal of R^1 if it is desired to obtain an oligonucleotide which does not have a 5'-protecting group.

The invention also relates to a method of preparing a trivalent phosphorus multimer represented by Structural Formula I, or a stereoisomer thereof. The method involves protecting a 3'-substituent of a nucleoside represented by Structural Formula VII, or a stereoisomer thereof:



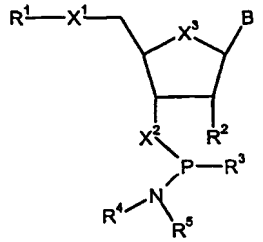
VII.

to form a first intermediate represented by Structural Formula VIII, or a stereoisomer thereof:



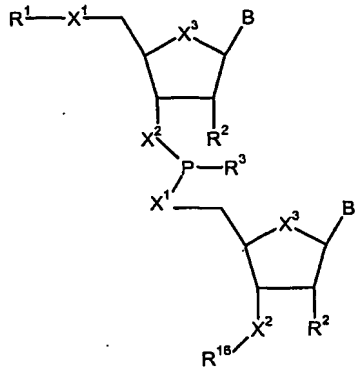
VIII.

In Structural Formulas VII and VIII, X^1, X^2, X^3, R^1 and B are as defined above. In Structural Formula VIII, R^{16} is a protecting group which is orthogonal to R^1. Therefore, R^1 is a protecting group that can be removed in the presence of R_{16} without reaction occurring at R^{16}, and R^{16} is a protecting group which can be removed in the presence of R^1 without reaction occurring at R^1. Preferably, R^{16} is a levulynoyl group or a 3-benzoylpropionyl group. The first intermediate represented by Structural Formula VIII is then treated to remove R^1 to form a 5'-deprotected nucleoside. When R^1 is an acid labile protecting group, the first intermediate is treated with an acid to remove the acid labile protecting group. When R^1 is a trialkylsilyl protecting group, the first intermediate is typically treated with fluoride ions to remove R^1. The 5'-deprotected nucleoside is reacted in the presence of a coupling catalyst with a compound represented by Structural Formula IX, or a stereoisomer thereof:



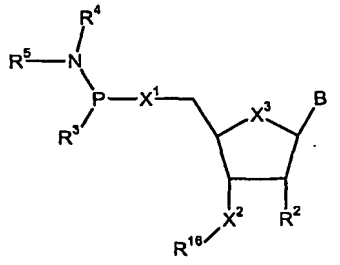
IX.

In Structural Formula IX, X^1, X^2, X^3, R^1, R^2, R^3, R^4, R^5 and B are as defined above. The 3',5'-protected dimer formed from the reaction can be represented by Structural Formula X, or a stereoisomer thereof:



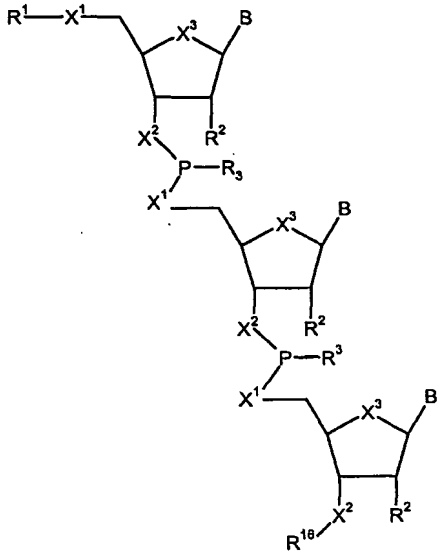
X.

In Structural Formula X, X^1, X^2, X^3, R^1, R^2, R^3, R^{16} and B are as defined above. The 3',5'-protected dimer is treated to remove R^{16} to form a 3'-deprotected dimer. The 3'-deprotected dimer is optionally reacted in the presence of a coupling catalyst with a compound represented by Structural Formula XI, or a stereoisomer thereof:



XI.

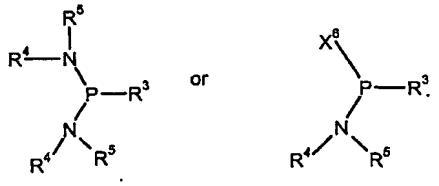
In Structural Formula XI, X^1, X^2, X^3, R^3, R^4, R^5, R^{16} and B are as defined previously. The product of the reaction is a 3',5'-protected trimer represented by Structural Formula XII, or a stereoisomer thereof:



XII.

In Structural Formula XII, X^1, X^2, X^3, R^1, R^2, R^3, R^{16} and B are as previously defined.

Optionally, the 3',5'-protected trimer can be treated to remove R^{16} to form a 3'-deprotected trimer which can be reacted in the presence of a coupling catalyst with a compound represented by Structural Formula XI one or more times to form a 3',5'-protected multimer. The 3',5'-protected multimer is then treated to remove R^{16} to form a 3'-deprotected multimer. The 3'-deprotected multimer is reacted with a trivalent phosphorus compound represented by either XIIIa or XIIIb:

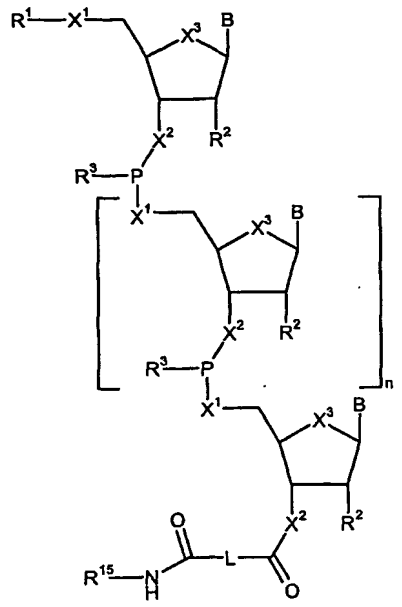


XIIIa XIIIb

to form a trivalent phosphorus multimer represented by Structural Formula I. In Structural Formulas XIIIa and XIIIb, R^3, R^4 and R^5 are defined as above. X^6 is a halogen, preferably chloro or bromo.

Alternatively, a trivalent phosphorus multimer represented by Structural Formula I, or a stereoisomer thereof can be prepared by protecting the 3'-substituent of a nucleoside represented by Structural Formula VII, or a stereoisomer thereof to form a first intermediate represented by Structural Formula VIII, or a stereoisomer thereof. The first intermediate is treated to remove R^1, thereby forming a 5'-deprotected nucleoside. The 5'-deprotected nucleoside is reacted with a trivalent phosphorus compound represented by either XIIIa or XIIIb to form a 5'-phosphoramidite represented by Structural Formula XI or a stereoisomer thereof. The 5'-phosphoramidite is reacted, in the presence of a coupling catalyst, with a compound represented by Structural Formula VII or a stereoisomer thereof to form a 3',5'-protected dimer represented by Structural Formula X or a stereoisomer thereof. The 3',5'-protected dimer is treated to remove R^{16} to form a 3'-deprotected dimer. Optionally, the 3'-deprotected dimer is reacted in the presence of a coupling catalyst with a compound represented by Structural Formula XI or a stereoisomer thereof to form a 3',5'-protected trimer represented by Structural Formula XII or a stereoisomer thereof. Optionally, removal of R^{16} and the coupling step can be repeated one more time to form a 3',5'-protected multimer. R^{16} is removed from the 3',5'-protected multimer to form a 3'-deprotected multimer which is reacted with a trivalent phosphorus compound represented by one of Structural Formulas XIIIa or XIIIb to form the trivalent phosphorus multimer represented by Structural Formula I or a stereoisomer thereof.

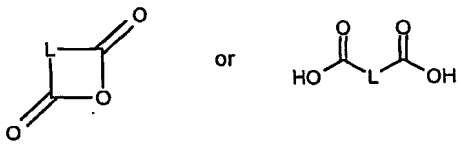
Another embodiment of the invention is a trivalent phosphorus multimer derivatized solid support and a method of preparing the same. The trivalent phosphorus multimer derivatized solid support can be represented by Structural Formula XIV or a stereoisomer thereof:



XIV.

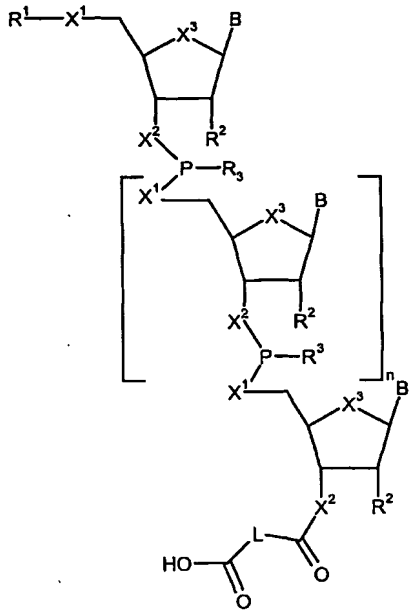
In Structural Formula XIV, X^1, X^2, X^3, R^1, R^2, R^3, R^{15}, B, L and n are as defined above.

The method of preparing the trivalent phosphorus multimer derivatized solid support represented by Structural Formula XIV involves preparing a 3'-deprotected multimer by one of the methods described above. The 3'-deprotected multimer is reacted in the presence of a base with a compound represented by Structural Formula XVa or XVb:



XVa or XVb

to form a solid support loading reagent represented by Structural Formula XVI or a stereoisomer thereof:



XVI.

In Structural Formulas XVa, XVb, and XVI, L is as defined above. In Structural Formula XVI, X^1, X^2, X^3, R^1, R^2, R^3, and B are as defined above. The solid support loading reagent is reacted with a solid support derivatized with primary or secondary amine functional groups in the presence of a base and a substituted or unsubstituted dialiphatic carbodiimide to form the trivalent phosphorus multimer derivatized solid support represented by Structural Formula XIV.

In certain embodiments, the stereoisomers of the compounds of Structural Formulae I to XII, XIV and XVI are the corresponding structural formulae in which the sugar moieties are the l-stereoisomers as opposed to the d-stereoisomers illustrated.

Because the multimers of the invention have trivalent phosphorus linkages, chimeric oligonucleotides (i.e., oligonucleotides that have both phosphodiester and phosphorothioate linkages) can be synthesized from the trivalent phosphorus multimers. The synthesis of oligonucleotides using trivalent phosphorus multimers is expected to improve the yield of the desired product because fewer reaction steps will be necessary than when monomers are utilized to synthesize oligonucleotides. Therefore, use of the trivalent phosphorus multimers of the invention is expected to facilitate the synthesis of longer oligonucleotides than is currently possible with available techniques. In addition, use of the multimers of the present invention will conserve reagents because half as many, or fewer, reaction steps are required to synthesize an oligonucleotide of a given length than when the synthesis is done using monomers. Finally, because N-1 side products are not likely to be produced when multimers are used to synthesize

oligonucleotides, the desired oligonucleotide will be more readily separated from failure sequences. Therefore, the desired product can be prepared with higher purity and in greater yield due to less loss of product during purification.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a synthetic scheme for a synthesis of a trivalent phosphorus multimer.

Figure 2 is an alternative synthetic scheme for a synthesis of a trivalent phosphorus multimer.

Figure 3 represents a 2'-O-methyl dimer prepared in Example 2.

Figure 4 represents two dimers utilized to prepare a phosphodiester and two phosphorothioates synthesized in Example 4.

Figure 5 is a synthetic scheme for the synthesis of oligonucleotides synthesized in Example 4 using trivalent phosphorus dimers. It will be recognised that the thymidine moiety shown bonded to the solid support may be replaced with alternative nucleosidic moieties as appropriate for the sequence desired to be manufactured.

DETAILED DESCRIPTION OF THE INVENTION

Aliphatic groups, as used herein, include straight chained or branched C_1-C_{18} hydrocarbons which are completely saturated or which contain one or more non-aromatic double bonds, or cyclic C_3-C_{18} hydrocarbons which are completely saturated or which contain one or more unconjugated double bonds. Lower alkyl groups are straight chained or branched C_1-C_8 hydrocarbons or C_3-C_8 cyclic hydrocarbons which are completely saturated. Aliphatic groups are preferably lower alkyl groups.

Aromatic groups include carbocyclic aromatic ring systems (e.g., phenyl) and carbocyclic aromatic ring systems fused to one or more carbocyclic aromatic or non-aromatic ring (e.g., naphthyl, anthracenyl and 1,2,3,4-tetrahydronaphthyl).

Heteroaromatic groups, as used herein, include heteroaryl ring systems (e.g., thienyl, pyridyl, pyrazole, isoxazolyl, thiadiazolyl, oxadiazolyl, indazolyl, furans, pyrroles, imidazoles, pyrazoles, triazoles, pyrimidines, pyrazines, thiazoles, isoxazoles, isothiazoles, tetrazoles, or oxadiazoles) and heteroaryl ring systems in which a carbocyclic aromatic ring, carbocyclic non-aromatic ring, heteroaryl ring or a heterocycloalkyl ring is fused to one or more other heteroaryl rings (e.g., benzo(b)thienyl, benzimidazole, indole, tetrahydroindole, azaindole, indazole, quinoline, imidazopyridine, purine, pyrrolo[2,3-d]pyrimidine, and pyrazolo[3,4-d]pyrimidine).

An aralkyl group, as used herein, is an aromatic substituent that is linked to a moiety by an aliphatic group preferably having from one to about six carbon atoms.

A heterocycloalkyl group, as used herein, is a non-aromatic ring system that preferably has 5 to 6 atoms and includes at least one heteroatom, such as nitrogen, oxygen, or sulfur. Examples of heterocycloalkyl groups include morpholines, piperidines, and piperazines.

Suitable substituents for aliphatic groups, aromatic groups, aralkyl groups, heteroaromatic groups and heterocycloalkyl groups include aromatic groups, halogenated aromatic groups, lower alkyl groups, halogenated lower alkyl (e.g. trifluoromethyl and trichloromethyl), -O-(aliphatic group or substituted aliphatic group), -O-(aromatic group or substituted aromatic group), benzyl, substituted benzyl, halogens, cyano, nitro, -S-(aliphatic or substituted aliphatic group), and -S-(aromatic or substituted aromatic).

Amine, alcohol and thiol protecting groups are known to those skilled in the art. For examples of amine protecting groups see Greene, *et al.*, *Protective Groups in Organic Synthesis* (1991), John Wiley & Sons, Inc., pages 309-405, the teachings of which are incorporated herein by reference in their entirety. Preferably, amines are protected as amides or carbamates. For examples of alcohol protecting groups see *Id.*, pages 10-142, the teachings of which are incorporated herein by reference in their entirety. A preferred alcohol protecting group is *t*-butyldimethylsilyl group. For examples of thiol protecting groups see *Id.*, pages 277-308, the teachings of which are incorporated herein by reference in their entirety.

An acid labile protecting group is a protecting group which can be removed by contacting the group with a Bronsted or a Lewis acid. Acid labile protecting groups are known to those skilled in the art. Examples of common acid labile protecting groups include substituted or unsubstituted trityl groups (*Id.*, pages 60-62), substituted or unsubstituted tetrahydropyranyl groups (*Id.*, pages 31-34), substituted or unsubstituted tetrahydrofuranyl groups (*Id.*, pages 36-37) or pixyl groups (*Id.*, page 65). A preferred acid labile protecting group is a substituted or unsubstituted trityl, for example 4,4'-dimethoxytrityl (hereinafter "DMT"). Trityl groups are preferably substituted by electron donating substituents such as alkoxy groups.

Nucleoside bases include naturally occurring bases, such as adenine, guanine, cytosine, thymine, and uracil and modified bases such as 7-deazaguanine, 7-deaza-8-azaguanine, 5-propynylcytosine, 5-propynyluricil, 7-deazaadenine, 7-deaza-8-azaadenine, 7-deaza-6-oxopurine, 6-oxopurine, 3-deazaadenosine, 2-oxo-5-methylpyrimidine, 2-oxo-4-methylthio-5-methylpyrimidine, 2-thiocarbonyl-4-oxo-5-methylpyrimidine, 4-oxo-5-methylpyrimidine, 2-amino-purine, 5-fluorouricil, 2,6-diaminopurine, 8-aminopurine, 4-triazolo-5-methylthymine, and 4-triazolo-5-methyluricil.

A protected nucleoside base is a nucleoside base in which reactive functional groups of the base are protected. Typically, nucleoside bases have amine groups which can be protected with an amine protecting group, such as by the formation of an amide or a carbamate group. For example, the amine groups of adenine and cytosine are typically

protected with benzoyl protecting groups, and the amine groups of guanine is typically protected with an isobutyryl group, an acetyl group or t-butylphenoxyacetyl group. However, other protection schemes may be used. For example, for fast deprotection, the amine groups of adenine and guanine are protected with phenoxyacetyl groups and the amine group of cytosine is protected with an isobutyryl group. When an oligonucleotide is synthesized from multimers of the present invention having protected nucleotide bases, conditions for removal of the protecting group will depend on the protecting group used. When an amino group protected as an amide group is used, it can be removed by treating the oligonucleotide with a base solution, such as a concentrated ammonium hydroxide solution, *n*-methylamine solution or a solution of *t*-butylamine in ammonium hydroxide.

References in this section to Structural Formulae will be understood to include the corresponding stereoisomers as appropriate.

A trivalent phosphorus multimer represented by Structural Formula I or II can be used to prepare an oligonucleotide represented by Structural Formula III. An advantage of using trivalent phosphorus multimers over using monomers is that fewer reaction steps are necessary. Therefore, longer oligonucleotides can be synthesized. It is expected that oligonucleotides longer than 150 nucleotide bases can be synthesized using trivalent phosphorus multimers. The multimers of the present invention can also be employed to synthesise shorter oligonucleotides, such as those comprising up to about 50 bases, for example from 8 to 35 bases.

The synthesis of the oligonucleotide can be done in solution or on a solid support. When the synthesis is in solution, R^{13} is an alcohol, amine or thiol protecting group. After synthesis of the oligonucleotide the alcohol, amine or thiol protecting group can be removed. When the oligonucleotide is synthesized on a solid support, R_{13} represents a solid support or $-Y^2-L-Y^2-R^{15}$. In general, the solution phase synthesis or the solid phase synthesis of oligonucleotides using trivalent phosphorus multimers are carried out similar to method which have been developed for synthesis of oligonucleotides from monomers, except that when a multimer is used, the coupling step often takes about 25% to about 75% longer than the coupling step takes when a monomer is used. Examples of typical conditions for solution phase synthesis and solid phase synthesis oligonucleotides using trivalent phosphorus multimers are set forth below.

The first step of preparing the oligonucleotide involves coupling the trivalent phosphorus multimer with a 5'-deprotected nucleoside or nucleotide represented by Structural Formula IV. During the coupling reaction, the 5'-deprotected group of the nucleoside or nucleotide reacts with the multimer by displacing the $-NR_4R_5$ group. When the synthesis is done in solution, the 5'-deprotected nucleotide is often present in a concentration of about 0.02 M to about 2 M, and the multimer is preferably present in a concentration of about 1.1 equivalents to about 2 equivalents of the 5'-deprotected nucleoside or nucleotide. About 2.5 equivalents to about 5.0 equivalents, with respect to

the 5'-deprotected nucleoside, of nucleophilic coupling catalyst, such as tetrazole, S-ethylthiotetrazole, dicyanoimidazole or pyridinium salts, such as pyridinium chloride, is usually added to facilitate the coupling reaction. The reaction time is commonly about 20 min. to about 60 min.

A second step of preparing an oligonucleotide involves oxidizing or sulfurizing the trivalent phosphorus groups of the oligonucleotide. In this step, the trivalent phosphorus groups of the multimer which has been coupled to the 5'-deprotected nucleoside, as well as the newly formed trivalent phosphorus linkage between the 5'-deprotected nucleoside or nucleotide and the multimer, is oxidized or sulfurized.

In a solution phase synthesis, the oxidation reaction is often carried out by treating the oligonucleotide with an oxidizing agent, for example, I_2 in the presence of water or a peroxide such as *t*-butyl hydrogen peroxide in an organic solvent. When I_2 and water are used, the oxidizing solution typically contains about 1.1 to about 1.8 equivalents of I_2 in the presence of a base and a trace amount of water. The reaction is carried out in an aprotic polar solvent, such as THF, combined with a base, such as a tertiary alkylamine and about 1% water. The ratio of aprotic solvent to base is about 4 : 1 (vol./vol.) to about 1 : 4 (vol./vol.). After about 5 min. to about 20 min., the reaction mixture is poured into an aqueous solution of sodium bisulfite to quench the excess iodine, then extracted into an organic solvent.

Alternatively, the trivalent phosphorus groups can be sulfurized using any sulfur transfer reagent known to those skilled in the art for use in oligonucleotide synthesis. Examples of sulfur transfer agents include *3H*-benzodithiol-3-one 1,1-dioxide (also called "Beaucage reagent"), dibenzoyl tetrasulfide, phenylacetyl disulfide, N,N,N',N'-tetraethylthiuram disulfide, 3-amino-[1,2,4]-dithiazole-5-thione (see US patent no. 6,096,881 the entire teachings of which are incorporated herein by reference), or elemental sulfur. Examples of reaction conditions for sulfurization of an oligonucleotide using the above reagents can be found in Beaucage, *et al.*, *Tetrahedron* (1993), *49*:6123, the teachings of which are incorporated herein by reference in their entirety. 3-amino-[1,2,4]-dithiazole-5-thione is a preferred sulfur transfer reagent. Generally, an oligonucleotide is contacted with a solution of 3-amino-[1,2,4]-dithiazole-5-thione in an organic solvent, such as pyridine/acetonitrile (1:9 w/w), having a concentration of about 0.04 M to about 0.2 M. The sulfurization reaction is complete in about 30 sec. to about 2 min.

After oxidation or sulfurization of the oligonucleotide, any unreacted 5'-deprotected nucleoside is often capped so that it cannot react in subsequent coupling steps. Capping failure sequences allows them to be more readily separated from full length oligonucleotide product. Any reagent which will react with the nucleophilic 5'-end group (i.e., -OH, -SH or $-NH_2$) and prevent it from reacting with a trivalent phosphorus multimer can be used as a capping reagent. Typically, an anhydride, such as acetic anhydride or

isobutyric anhydride, or an acid chloride, such as acetyl chloride or isobutyryl chloride, in the presence of a base is used as a capping reagent.

After the capping reaction is complete, the 5'-protecting group, represented by R^1 is removed. When R_1 is an acid labile protecting group, R^1 is removed by treating the oligonucleotide with an acid. Preferably, the 5'-protecting group is a trityl group, such as 4,4'-dimethoxytrityl. When the 5'-protecting group is a trityl group, it can be removed by treating the oligonucleotide with a solution of dichloroacetic acid or trichloroacetic acid in an organic solvent, such as dichloromethane. Once the 5'-protecting group has been removed, the reaction cycle (i.e., coupling step, oxidation or sulfurization step, capping step (optional) and deprotection step) optionally can be repeated one or more times to obtain an oligonucleotide of the desired length.

A phosphate oligonucleotide can be prepared by oxidising the trivalent phosphorus groups and by selection of the internucleotide phosphorus protecting groups (such as represented by R^3) such that when the internucleotide groups are deprotected, phosphate groups are formed. For example, oxidation of a beta-cyanoethyloxy protected trivalent phosphorus followed by basic deprotection would form a phosphate group.

A phosphorothioate oligonucleotide can be prepared by sulfurizing the trivalent phosphorus groups or by oxidising the trivalent phosphorus groups and by selection of the internucleotide phosphorus protecting groups (such as represented by R^3) such that when the internucleotide groups are deprotected, phosphorothioate groups are formed. For example, oxidation of a beta-cyanoethylthio protected trivalent phosphorus followed by anhydrous basic deprotection would form a phosphorothioate group.

A chimeric oligonucleotide can be prepared by oxidizing the trivalent phosphorus groups in one or more reaction cycles and sulfurizing the trivalent phosphorus groups in one or more different reaction cycles, combined with appropriate selection of the internucleotide phosphorus protecting groups (such as represented by R^3) to form the desired phosphate or phosphorothioate linkage. Alternatively, a chimeric oligonucleotide can be prepared by selecting multimers in which some of the internucleotide protecting groups form phosphorothioate linkages on deprotection, such as beta-cyanoethylthio protecting groups, and some of the internucleotide protecting groups form phosphate linkages on deprotection, such as beta-cyanoethyloxy protecting groups. In this method, the oligonucleotide is oxidized after the coupling step in each reaction cycle.

When it is desired to obtain an oligonucleotide product in which the 5'-end group is protected, the final step of the reaction cycle can be the capping step, if a capping step is done, or the final step of the reaction can be an oxidation or sulfurization step if a capping step is not done. If a 5'-deprotected oligonucleotide is desired, the reaction cycle can end with the deprotection step. Usually, a 5'-protected oligonucleotide is the desired product if the oligonucleotide is to be purified by reverse phase high performance liquid chromatography (HPLC). If the oligonucleotide is to be purified by ion-exchange

chromatography, a 5'-deprotected oligonucleotide is usually the desired product.

The solid phase synthesis of an oligonucleotide using trivalent phosphorus multimers generally utilizes the same reaction cycle and reagents as the solution phase synthesis. However, the 5'-deprotected nucleoside or nucleotide is loaded on the solid support, where loading is often about 50 µmole to about 100 µmole per gram of support. In the coupling step, a solution of trivalent phosphorus multimer, typically having a concentration of about 0.01 M to about 1 M, preferably about 0.1 M, in an organic solvent, such as acetonitrile, is added to the solid support. A solution of the nucleophilic coupling catalyst having a concentration of about 1.5 mmol to about 1.5 M, is usually mixed with the solution containing the multimer just prior to or during the coupling reaction. Then the support bound 5'-deprotected nucleoside or nucleotide is contacted with the mixture for about 2 min. to about 10 min., preferably about 5 min.

If the trivalent phosphorus linkages are to be oxidized after the coupling reaction is complete, the solid support is contacted with an oxidizing agent such as a mixture of I_2 and water or a peroxide such as *t*-butyl hydroperoxide in an organic solvent such as THF, acetonitrile or toluene. A mixture of I_2 and H_2O is a preferred oxidizing reagent. When a mixture of I_2 and water is used other water miscible organic solvents can also be present. Typically, the solid support bound oligonucleotide containing trivalent phosphorus internucleotide linkages can be contacted with a solution of I_2 in a solvent mixture of water, an aprotic, water miscible solvent, and a base. An example of a typical oxidation solution is about 0.05 M to about 1.5 M I_2 in a solution of (2:80:20) water/tetrahydrofuran/lutidine (vol./vol./vol.). The solid support is typically treated with the I_2 solution for about 30 seconds to about 1.5 min.

Alternatively, the solid support can be contacted with a solution of a sulfur transfer reagent in an organic solvent to sulfurize the trivalent phosphorus groups. For example, the solid support can be contacted with a solution of *3H*-benzodithiol-3-one-1,1-dioxide (about 0.05 M-0.2 M) in an organic solvent, such acetonitrile, for about 30 sec. to about 1 min.

In solid phase oligonucleotide synthesis, the solid support optionally can be contacted with a solution of the capping reagent for about 30 sec. to about 1 min. Following the capping step, the deprotection step is accomplished by contacting the solid support with an acid solution for about 1 min. to about 3 min. The reaction cycle can optionally be repeated one or more times until an oligonucleotide of the desired length is synthesized. As in the solution phase synthesis, a 5'-protected oligonucleotide is obtained when the reaction cycle ends with either the capping step or the oxidation or sulfurization step. A 5'-deprotected oligonucleotide is obtained when the reaction cycle is ended with the deprotection step.

When the solid phase synthesis is completed, the oligonucleotide can be removed from the solid support by standard methods. Generally, the solid support is treated with a

solution of concentrated ammonium hydroxide at 25°C-60°C for about 0.5 hours to about 16 hours or longer depending on the oligonucleotide sequence and whether it is desired to remove the nucleobase protecting groups during this step.

The trivalent phosphorus multimers can be prepared by two different methods. In the first method (for an example of this method see Figure 1), a nucleoside represented by Structural Formula VII is protected to form a first intermediate represented by Structural Formula VIII. Preferably, the protecting group is a levulynoyl protecting group or a 3-benzoylpropionyl protecting group. The levulynoyl protecting group can be added to the nucleoside by several different methods (see Greene, *et al.*, *Protective Groups in Organic Synthesis* (1991), John Wiley & Sons, Inc., page 97, the teachings of which are incorporated herein by reference in their entirety). A preferred method for protecting the nucleoside with a levulynoyl or 3-benzoylpropionyl protecting group is by treating it with levulinic anhydride or 3-benzoylpropionic anhydride in pyridine for about 16-24 hours. Alternatively, the levulynoyl or 3-benzoylpropionyl protecting group can be added by treating the nucleoside with levulinic acid or 3-benzoylpropionic acid and dicyclohexylcarbodiimide in a solution of dichloromethane and pyridine.

The first intermediate is then treated to remove R_1 to form a 5'-deprotected nucleoside. Methods for removing the trityl group are listed in Greene, *et al.*, *Protective Groups in Organic Synthesis* (1991), John Wiley & Sons, Inc., pages 60-62, the teachings of which are incorporated herein by reference in their entirety. Methods for removing a tetrahydropyranyl protecting group (pages 31-34) and tetrahydrofuranyl protecting group (pages 36-37) can be found in Greene, *et al.*, *Protective Groups in Organic Synthesis* (1991), John Wiley & Sons, Inc., the teachings of which are incorporated herein by reference in their entirety. A trityl group is preferably removed by treating the first intermediate with an aqueous solution of 80% (vol./vol.) acetic acid in water for about 7 hours, or by treatment with about 2% trifluoroacetic acid or dichloroacetic acid in dichloromethane for about 15 minutes.

The 5'-deprotected nucleoside can then be coupled with a phosphoramidite represented by Structural Formula IX to form a dimer represented by Structural Formula X. The coupling reaction is typically carried out in an organic solvent under dry reaction conditions. The concentration of the reactants is about 0.05 M to about 0.5 M. A coupling catalyst, such as *H*-tetrazole or *S*-ethylthiotetrazole, is also present in the reaction mixture, often in about 2 equivalents to about 6 equivalents with respect to the 5'-deprotected nucleoside. The coupling reaction typically takes about 2 hours to about 16 hours.

The 3',5'-protected dimer is then treated to remove R^{16} to form a 3'-deprotected dimer. When R^{16} is a levulynoyl protecting group, it can be removed by the methods set forth in Greene, *et al.*, *Protective Groups in Organic Synthesis* (1991), John Wiley & Sons, Inc., p. 97, the teachings of which are incorporated herein by reference in their entirety.

Preferably, the levulynoyl or 3-benzoylpropionyl protecting group is removed by contacting the 3',5'-protected dimer with a solution of about 0.05 M to about 1 M hydrazine in pyridine/acetic acid in a ratio of about 4 : 1 (vol./vol.) to about 1 : 4 (vol./vol.) for about 15 min. to about 1 hour.

The 3'-deprotected dimer is optionally reacted in the presence of a coupling catalyst with a compound represented by Structural Formula XI. This coupling reaction is carried out in an organic solvent under dry reaction conditions. The concentration of the 3'-deprotected dimer and the compound represented by Structural Formula XI is about 0.05M to about 0.5M. A coupling catalyst, such as *H*-tetrazole or *S*-ethylthiotetrazole, is present in about 2 equivalents to about 6 equivalents with respect to the 3'-deprotected dimer. A 3',5'-protected trimer represented by Structural Formula XII is the product formed by the reaction.

The R^{16} deprotection reaction and the coupling reaction can optionally be repeated one or more times using the 3',5'-protected trimer as the starting material to form a 3',5'-protected multimer.

The 3',5'-protected multimer is then treated to remove R^{16} to form a 3'-deprotected multimer.

The 3'-deprotected multimer is reacted with a phosphorylating reagent to form the trivalent phosphorus multimer represented by Structural Formula I. A phosphorylating reagent represented by Structural Formula XIIIa or XIIIb is added slowly to a dry solution of the 3'-deprotected multimer in an organic solvent, such as dichloromethane, acetonitrile or THF. When the phosphorylating reagent is a compound represented by Structural Formula XIIIb, an aprotic organic base, such as triethylamine or diisopropylethylamine, is also added to the reaction mixture. The phosphorylating reagent is often present in an excess of about 1.1 equivalents to about 1.5 equivalents with respect to the multimer. The reaction is allowed to proceed for about 4 hours to about 24 hours. After the reaction is complete, n-octanol optionally can be added to the reaction mixture to react with any remaining phosphorylating reagent. Preferably, multimers are dimers (two nucleotide bases) or trimers (three nucleotide bases).

In the second method of preparing a trivalent phosphorus multimer (for an example see Figure 2), a nucleoside base represented by Structural Formula VII is protected with R^{16}, a protecting group which is orthogonal to R^1, to form a first intermediate represented by Structural Formula VIII. Preferably, R^{16} is a levulynoyl or a 3-benzoylpropionyl protecting group. The first intermediate is treated to remove R^1 in the same manner as in the first method of preparing a trivalent phosphorus multimer. The 5'-deprotected nucleoside formed by this reaction sequence is treated with a phosphorylating reagent represented by Structural Formula XIIIa or XIIIb to form a 5'-phosphoramidite nucleoside represented by Structural Formula XI. The 5'-phosphorylation conditions are the same as described for the 3'-phosphorylation

conditions of the first method of forming a trivalent phosphorus multimer. The 5'-phosphoramidite is then coupled with a compound represented by Structural Formula VII in the presence of a coupling catalyst to form a 3',5'-protected dimer represented by Structural Formula X. The reaction conditions for the coupling reaction are the same as for the coupling reaction in the first method of forming the multimer. The 3',5'-protected dimer is treated to remove R^{16} as described above to form a 3'-deprotected dimer. Also as described above, the 3'-deprotected dimer can optionally be reacted in the presence of a coupling catalyst with a compound represented by Structural Formula XI to form a 3',5'-protected trimer represented by Structural Formula XII. The 3'-deprotection reaction and the coupling reaction can be optionally repeated one or more times to form a 3',5'-protected multimer. R_{16} is removed from the 3',5'-protected multimer to form a 3'-deprotected multimer, and the 3'-deprotected multimer is phosphorylated as described above to form a trivalent phosphorus multimer represented by Structural Formula I.

A solid support which is derivatized with a trivalent phosphorus multimer represented by Structural Formula XII can be prepared by reacting the 3'-deprotected multimer, formed by the method described above, with a compound represented by Structural Formula XVa or XVb to form a solid support loading reagent. When the 3'-deprotected multimer is reacted with a compound represented by Structural Formula XVa, the reaction is carried out in the presence of a base, such as pyridine. When the 3'-deprotected multimer is reacted with a compound represented by Structural Formula XVb, at least about 1 equivalent of a dialiphatic carbodiimide, with respect to the compound represented by Structural Formula XVb, should also be present in the reaction mixture. The solid support loading reagent optionally can be activated by reacting it with a compound which will transform the terminal carboxylic acid into an active ester. Examples of compounds which will react with the terminal carboxylic acid to form an active ester include *p*-nitrophenol, *o,p*-dinitrophenol, imidazole, and N-hydroxysuccinamide. In the reaction to form an active ester with *p*-nitrophenol or *o,p*-dinitrophenol, an aliphatic carbodiimide should also be present in the reaction mixture.

The solid support loading reagent or the activated solid support loading reagent can be utilized to derivatize an amine functionalized solid support. Amine functional groups on the solid support can be primary or secondary amines. The amine functionalized support is contacted with the activated solid support loading reagent in a basic solution to form the derivatized solid support. For an example of reaction conditions see U.S. Patent No. 5,668,268, the teachings of which are incorporated herein in their entirety. Alternatively, the amine functionalized solid support is contacted with the unactivated loading reagent in the presence of a dialiphatic carbodiimide in a basic solution to derivatize the solid support. For an example of reaction conditions see U.S. Patent No. 5,668,268 and U.S. Patent No. 4,812,512, the teachings of which are incorporated herein by reference in their entirety. Preferred dialiphatic carbodiimides are

dicyclohexyl carbodiimide and diisopropyl carbodiimide.

EXAMPLES

Phosphoramidite dimers were prepared using the procedures set forth in Examples 1 and 2. A procedure for preparing phosphoramidite trimers is set forth in Example 3. Oligonucleotides having phosphodiester and/or phosphorothioate linkages were prepared using the method detailed in Example 4. In Examples 1-4, all parts and percentages are by weight unless otherwise specified.

EXAMPLE 1 (see Figure 1):

A. Synthesis of 2'-deoxy-N^4-benzoyl-3'-O-levulynoyl-cytidine:

The title compound was prepared from commercially available 2'-deoxy-N^4-benzoyl-5'-O-DMT-cytidine. 2'-deoxy-N^4-benzoyl-5'-O-DMT-cytidine was treated at room temperature for 5 hrs. with levulinic acid anhydride (1.5 eq.) in dry pyridine to give 2'-deoxy-N^4-benzoyl-3'-O-levulynoyl-5'-O-DMT-cytidine. The 5'-O-DMT protecting group was removed by subjecting 2'-deoxy-N^4-benzoyl-3'-O-levulynoyl-5'-O-DMT-cytidine to 80% (vol./vol.) acetic acid in water for 1.5 hrs. at room temperature to yield 2'-deoxy-N^4-benzoyl-3'-O-levulynoyl-cytidine. The product thus obtained was purified by silica gel column chromatography.

B. Synthesis of 2'-deoxy-5'-O-DMT-N^2-isobutyryl-guanosine-3'-O-P-(OCH_2CH_2CN)-5'-O-N^4-benzoyl-2'deoxycytidine-3'-O-levulinate:

N^4-Benzoyl-2'-deoxycytidine-3'-O-levulinate (8.58g, 20mmol) synthesized in step A and tetrazole (0.56g, 80mmol) were dried by co-evaporation with dry toluene under reduced pressure then dissolved in dry acetonitrile (100 mL). Commercially available N^2-isobutyryl-5'-O-DMT-2'deoxyguanosine-3'-O-phosphoramidite (16.0g, 19mmol) was added to this solution and the solution was allowed to stir under argon for 4hr at room temperature. The solution was concentrated to a viscous mass, then taken up in ethyl acetate saturated with argon gas. This solution was washed with a cold sodium bicarbonate solution followed by cold water. After drying over anhydrous sodium sulfate, the solution was concentrated under reduced pressure to light yellow foam. The product was purified by column chromatography to yield fully protected dimer nucleosides phosphite triester (19.5g) which was characterized by NMR.

C. Synthesis of 2'-deoxy-5'-O-DMT-N^2-isobutyryl-guanosine-3'-O-P-(OCH_2CH_2CN)-5'-O-N^4-benzoyl-2'deoxycytidine-3'-OH:

The fully protected di-nucleosides phosphite triester (17.5g, 15mmol) synthesized in step B was treated with hydrazine solution (0.5M, 100ml) in pyridine/acetic acid (4:1 vol./vol.) for 30 min at room temperature. Pyridine (100ml) was added to the solution, and the mixture was concentrated under reduced pressure. The product was taken up in dichloromethane (500ml), then carefully washed with sodium bicarbonate solution (2 x 300 ml) and then with water (300 ml). The organic layer was dried over anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure to solid foam. The solid foam was then purified by silica gel chromatography to yield the desired product (13.4g, 83.4%).

D. Synthesis of 2'-deoxy-5'-O-DMT-N^2-isobutyryl-guanosine-3'-O-P-(OCH_2CH_2CN)-5'-O-N^4-benzoyl-2'deoxycytidine-3'-O-β-cyanoethyl-(N,N-diisopropylamino)-phosphoramidite:

The 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-O-P-(OCH_2CH_2CN)-5'-O-N^4-benzoyl-2'deoxycytidine-3-OH (12.5g, 11.7mmol) synthesized in step C was dried by co-evaporating with acetonitrile under reduced pressure. The dried material was dissolved in distilled dichloromethane. Bis-(N,N-diisopropylamino)-β-cyanoethoxyphosphine (4.5g, 15mmol) was added to the solution using a syringe, and S-ethylthiotetrazole (1.6g, 12.5mmol) was added as a solid. After stirring at room temperature for 16hr, n-octanol (500 l) was added, and the solution was stirred for another 2 hr. The reaction mixture was concentrated to a viscous liquid which was dissolved in ethyl acetate (500ml), then washed with a sodium bicarbonate solution followed by an sodium chloride solution. The ethyl acetate solution was drying over anhydrous sodium sulfate, then concentrated to a solid. The purification of the crude product was performed by silica gel column chromatography, which afforded desired dimer phosphoramidite (12.8g, 86.2%).

EXAMPLE 2:

A. Synthesis of 2'-deoxy-2'-O-methyl-3'-O-levulynoyl-uridine:

2'-Deoxy-2'-O-methyl-5'-O-dimethoxytrityl-uridine (11.2g, 20mmol) was dried by co-evaporation with pyridine (3 x 100 ml) then dissolved in dry pyridine (150ml). Levulinic acid anhydride (6.5g, 30mmol) and N,N-dimethylaminopyridine (0.1g, catalytic amount) were added and the solution was allowed to stir at room temperature for 5 hrs. When no starting material, as evaluated by tlc, remained in the solution, the solution was concentrated down to a viscous mass, then taken up in ethyl acetate. The ethyl acetate solution was washed with a sodium bicarbonate solution followed by a sodium chloride solution. The ethyl acetate layer was dried over sodium sulfate, then concentrated to

foam to give 2'-deoxy-2'-O-methyl-3'-O-levulynoyl-5'-O-dimethoxytrityl-uridine was characterized by proton NMR. This material was used in the next step without purification.

2'-Deoxy-2'-O-methyl-3'-O-levulynoyl-5'-O-dimethoxytrityl-uridine (14.0g) was then treated with 80% (vol./vol.) acetic acid solution in water (500 ml) for 1.5 hrs at room temperature. Pyridine (400 mL) was added to this solution, and the mixture was concentrated to viscous liquid under reduced pressure. The viscous mass was triturated with ether to provide a gummy solid, which was purified by silica gel column chromatography using ethyl acetate with increasing percentage of methanol. The desired fractions were pooled and concentrated to a solid. 2'-Deoxy-2'-O-methyl-3'-O-levulynoyl-uridine crystallized from a methylene chloride and ether mixture. The product was characterized by proton NMR.

B. Synthesis of 2'-deoxy-2'-O-methyl-5'-O-DMT-uridine-3'-O-P-(OCH_2CH_2CN)-5'-O-uridine-2'-deoxy-2'-O-methyl-3'-O-levulinate (coupling reaction):

2'-Deoxy-2'-O-methyl-3'-O-levulynoyl-uridine (5.0g, 14.0mmol) synthesized in step A and tetrazole were dissolved in dry acetonitrile and co-evaporated under reduced pressure. The dried material was pumped down with a high vacuum pump for 1 hr then dissolved in acetonitrile (70ml). 2'-Deoxy-2'-O-methyl-5'-O-DMT-uridine-3'-phosphoramidite (9.5g, 12.5mmol) was added to the solution and the reaction mixture was allowed to stir for 16 hr at room temperature. The organic solvent was removed under reduced pressure, and residue was taken up in ethyl acetate which has been saturated with argon. The solution was washed with a solution of sodium bicarbonate followed by a sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate, then concentrated after under reduced pressure. The crude product was purified by silica gel chromatography using ethyl acetate and increasing percentage of methanol. The desired pooled fractions were combined and concentrated to yield 9.5 g of the fully protected dimer. Finally the product was characterized by proton and phosphorus NMR.

C. Synthesis of 2'-deoxy-2'-O-methyl-5'-O-DMT-uridine-3'-O-P-(OCH_2CH_2CN)-5'-O-uridine-2'-deoxy-2'-O-methyl (removal of 3'-O-levulynoyl group):

The fully protected di-nucleoside phosphite triester (8.0g, 8.75 mmol) synthesized in step B was treated with a solution of hydrazine hydrate (0.5 M, 50 ml) in pyridine: acetic acid (4:1) for 30 min at room temperature. Pyridine (50ml) was added to the solution, then the mixture was concentrated to a viscous liquid. This material was then taken up in chloroform, then washed with a sodium bicarbonate solution and water. The chloroform extract was dried over sodium sulfate, then evaporated to dryness. The crude material

thus obtained was purified by silica gel column chromatography to give the 3'-deprotected dimer (6.5g, 8.0mmol). This was characterized by proton and phosphorus NMR.

D. Synthesis of 2'-deoxy-2'-O-methyl-5'-O-DMT-uridine-3'-O-P-(OCH_2CH_2CN)-5'-O-uridine-2'-deoxy-2'-O-methyl-3'-O-β-cyanoethyl-(N,N-diisopropylamino)-phosphoramidite (Dimer nucleoside phosphoramidite synthon):

The 3'-deprotected dinucleosides phosphite triester (6.1g, 7.46mmol) synthesized in step C was dried by co-evaporating with dry acetonitrile under reduced pressure. The dried material was dissolved in freshly distilled and dry methylene chloride (50ml). Phosphitylating reagent bis-(N,N-diisopropylamino)-β-cyanoethoxylphosphine (3.4ml, 11.2mmol) was added to the solution, followed by S-ethylthiotetrazole (0.75g, 6.0mmol), and the reaction mixture was stirred for 16 hr at room temperature. n-octanol (0.13ml) was added to the reaction mixture to react with excess phosphitylating reagent, and the reaction was stirred for another 2 hrs. Finally, the reaction mixture was diluted with 5% diisopropylethyl amine in chloroform (vol./vol.) (350ml) and washed immediately with a sodium bicarbonate solution (2 x 150 ml) and a sodium chloride solution (150 ml). The chloroform layer was dried over sodium sulfate, then concentrated to a solid. The dimer phosphoramidite product (see Figure 3) was purified by silica gel chromatography using 0.1% (vol./vol.) diisopropylethyl amine in ethyl acetate. 6.5 g of the desired pooled fractions was obtained in 98% purity by P-NMR.

EXAMPLE 3: Synthesis of trimer phosphoramidites:

A trimer phosphoramidite can be prepared by the reacting a phosphoramidite dimer nucleoside (e.g., the compound formed in Example 1D or 2D) with a 5'-hydroxy-2'-deoxy-3'-O-levulynoyl-nucleoside or a 5'-hydroxy-2'-O-protected-3'-O-levulynoyl-nucleoside in presence of 4.0 eq. of tetrazole in acetonitrile using reaction conditions similar to those given in Example 1B or 2B. Levulynoyl from 3'-end of the trimer-nucleoside can be removed by using hydrazine solution in pyridine and acetic acid using reaction conditions similar to those given in Example 1C or 2C. After purification, the 3'-deprotected trimer nucleosides with two phosphite triester linkages can be phosphitylated with bis-(N,N-diisopropylamino)-β-cyanoethoxy-phosphine and S-ethyl thio-tetrazole using reaction conditions similar to those given in Example 1D or 2D to give the phosphoramidite of trimer-nucleoside with two phosphite triester linkages.

EXAMPLE 4: Synthesis of an oligonucleotide having both phosphate diester and phosphorothioate diester linkages (see Figure 5):

Synthesis of the oligonucleotide was carried out on a DNA synthesizer 8909 Expedite (Applied Biosystems). The standard phosphoramidite chemistry protocol was followed for the synthesis with slight modifications. The concentration of phosphoramidite dimer nucleosides having phosphite triester linkages was 0.1M in acetonitrile. The dimers used for the synthesis are shown in Figure 4. The coupling time used for chain elongation using dimer phosphoramidites were 50% longer compared to monomer phosphoramidites. After coupling phosphite triester linkages were converted either to stable phosphate triester with iodine solution or to stable phosphorothioate with Beaucage reagent. Ten reaction cycles were performed in each synthesis to form an oligonucleotide with 21 nucleotide bases. A phosphate 21mer having the sequence ACACACACACACACACACACT (SEQ ID No. 1) and two phosphorothioate 21mers having the sequences ACACACACACACACACACACT (SEQ ID No. 1) and GTGTGTGTGTGTGTGTGTGTT (SEQ ID No. 2) were prepared. At the end of the synthesis, solid supports linked with fully protected oligonucleotide chain was treated with concentrated ammonium hydroxide for 16 hr at 50°C in order to release the chain and to remove the β-cyanoethyl protecting groups and the nucleoside base protecting groups. The crude oligonucleotides were analyzed by ion exchange HPLC. The main peak in the chromatogram was the desired product. In the case of oligonucleotide with phosphorothioated diester linkage, the crude product was also analyzed by phosphorus NMR. The spectra gave the characteristic chemical shift for phosphorothioate diester and no chemical shift for phosphate diester linkage could be seen.

EQUIVALENTS

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

1. A phosphoramidite compound comprising two or more nucleoside moieties linked by one or more internucleoside phosphorus atoms, wherein the internucleoside phosphorus atoms are phosphorus (III) atoms.

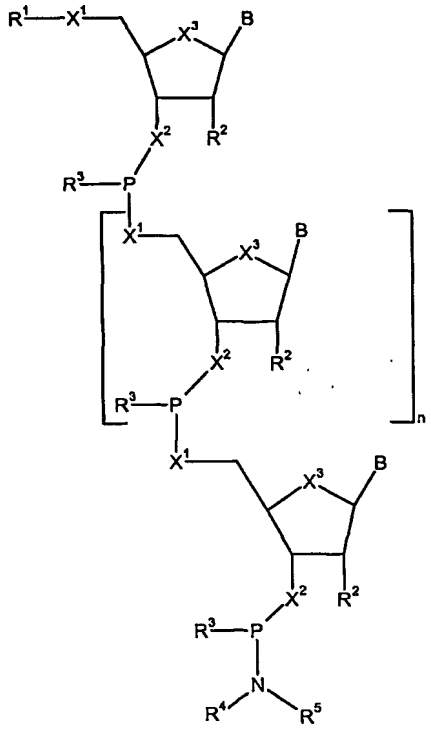
2. A phosphoramidite compound according to claim 1 which comprises a phosphoramidite moiety bonded to the 3'-position of the nucleoside moiety carrying the phosphoramidite moiety.

3. A phosphoramidite compound according to either of claims 1 and 2, wherein the phosphoramidite moiety is a group of formula $-X^1-PR^3NR^4R^5$ wherein R^3 represents a beta-cyanoethyloxy or beta-cyanoethylthio group and R^4 and R^5 represent isopropyl groups.

4. A phosphoramidite compound according to any preceding claim wherein the internucleoside phosphorus (III) atom comprises a phosphite triester group.

5. A phosphoramidite compound according to claim 4, wherein the phosphite triester group links the 5'-position of a nucleoside moiety carrying the phosphoramidite moiety with the 3'-position of a second nucleoside moiety.

6. A trivalent phosphorus multimer represented by the following structural formula, or a stereoisomer thereof:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or -NR-;

each X^3 is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$;

R^1 is a protecting group;

each R^2 is, independently, -H, -F, $-NHR^6$, $-CH_2R^6$ or $-OR^6$;

each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^7$, or $-SR^7$;

R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocycloalkyl group or a heteroaromatic group;

R is -H, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aromatic group, or an amine protecting group;

R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or a protecting group;

R^7 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group or a substituted or unsubstituted aralkyl;

each B is, independently, H or a protected or an unprotected nucleoside base;

and

n is 0 or a positive integer.

7. The trivalent phosphorus multimer of Claim 6, wherein each X^1, X^2 and X^3 is -O-.

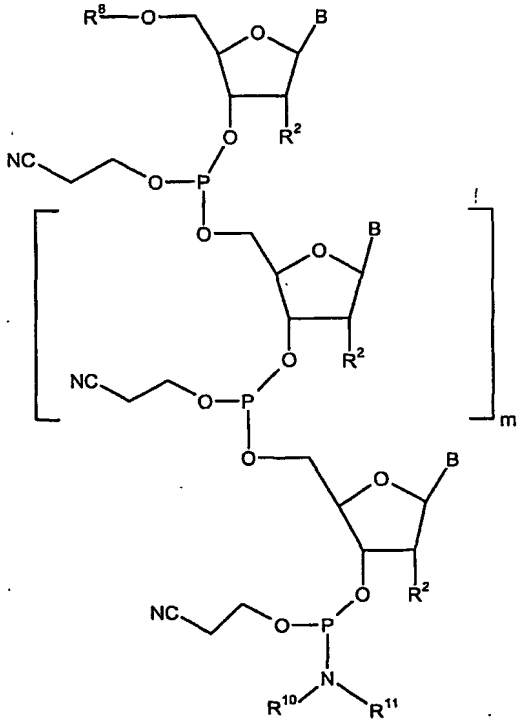
8. The trivalent phosphorus multimer of Claims 6 or 7, wherein R^3 is $-OCH_2CH_2CN$ or $-SCH_2CH_2CN$.

9. The trivalent phosphorus multimer of Claims 6, 7 or 8, wherein R^1 is an acid labile protecting group.

10. The trivalent phosphorus multimer of Claims 6, 7, 8 or 9, wherein R^2 is -H.

11. The trivalent phosphorus multimer of Claims 6, 7, 8 or 9, wherein R^2 is $-OR^6$ and R^6 is Me, $-CH_2CH_2OMe$ or a hydroxy protecting group.

12. A trivalent phosphorus multimer represented by the following structural formula, or a stereoisomer thereof:



wherein:

R^2 is -H or $-OR^6$;

R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or an alcohol protecting group;

R^8 is a substituted or unsubstituted trityl;

R^{10} and R^{11} are each, independently a substituted or unsubstituted aliphatic group;

each B is, independently, H or a protected or an unprotected nucleoside base; and

m is 0 or 1.

13. The multimer of Claim 12, wherein:

R^8 is 4,4'-dimethoxytrityl;

R^2 is -H; and

R^{10} and R^{11} are isopropyl.

14. The multimer of Claim 12, wherein:

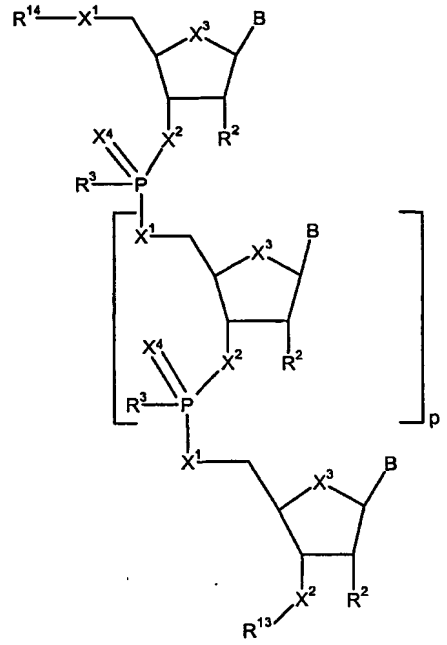
R^8 is 4,4'-dimethoxytrityl;

R^2 is $-OR^6$;

R^{10} and R^{11} are isopropyl; and

R^6 is Me, $-CH_2CH_2OMe$, *t*-butyldimethylsilyl, tetrahydropyranyl, 4-methoxy-tetrahydropyranyl or Fpmp.

15. A method of preparing an oligonucleotide represented by the following structural formula, or a stereoisomer thereof:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or -NR-;

each X^3 is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$;

each X^4 is, independently, =O or =S;

R is -H, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aromatic group, or an amine protecting group;

each R^2 is, independently, -H, -F, $-NHR^6$, $-CH_2R^6$ or $-OR^6$;

R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or a protecting group;

each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^7$, or $-SR^7$;

R^7 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group or a substituted or unsubstituted aralkyl;

R^{13} is an alcohol protecting group, an amine protecting group, a thiol protecting group, a group of the formula $-Y^2-L-Y^1$, a group of the formula $-Y^2-L-Y^2-R^{15}$ or a solid support;

R^{14} is -H or a protecting group;

each B is, independently, H or a protected or an unprotected nucleoside base;

p is a positive integer;

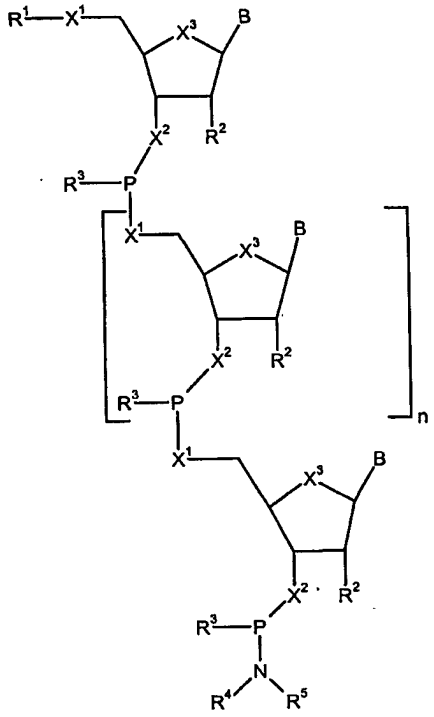
Y^1 is an ester or a carboxylic acid group;

Y^2 is a single bond, -C(O)-, -C(O)NR^{17}-, -C(O)O-, -NR^{17}- or -O-;

L is a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group; and

R^{17} is -H, a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group, comprising the steps of:

a) coupling a trivalent phosphorus multimer represented by the following structural formula, or a stereoisomer thereof:



wherein:

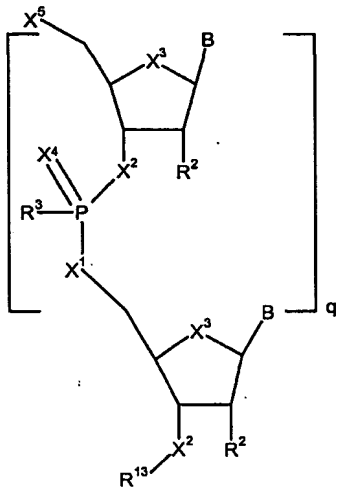
R^1 is a protecting group;

R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocycloalkyl group or a heteroaromatic group; and

n is 0 or a positive integer,

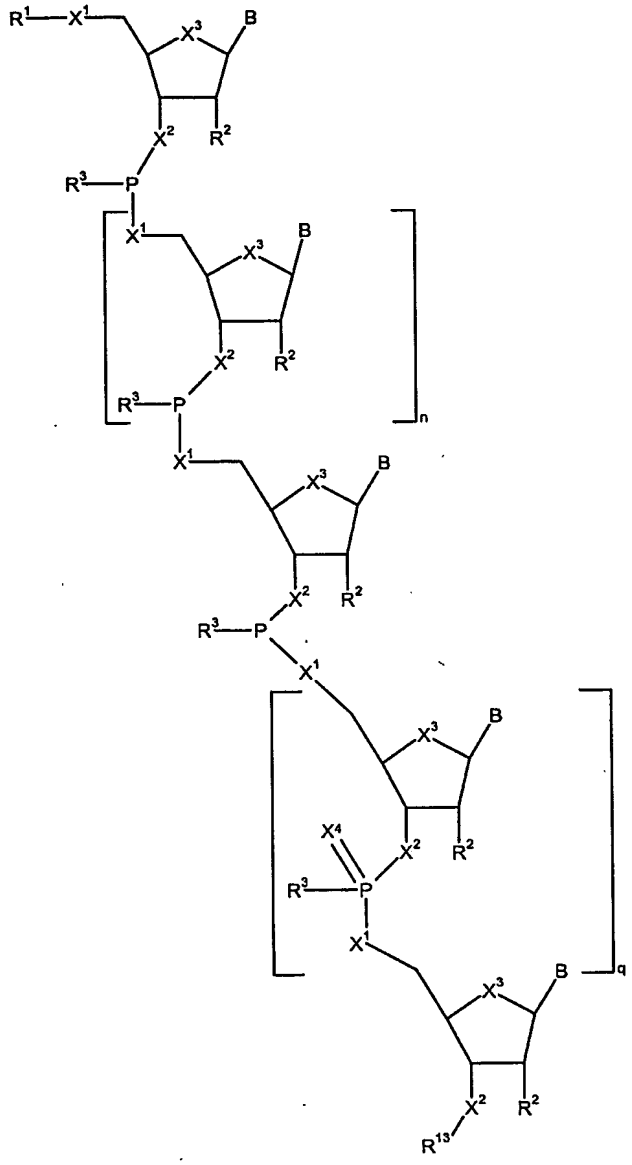
with a 5'-deprotected nucleoside or oligonucleotide represented by the following structural formula, or a stereoisomer thereof:



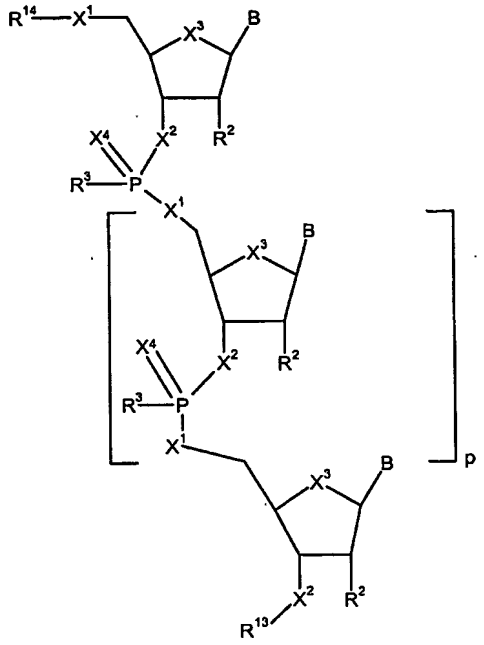
wherein:

X^5 is -OH or -SH; and

q is 0 or a positive integer, to produce a first intermediate represented by the following structural formula, or a stereoisomer thereof:



b) oxidizing or sulfurizing the trivalent phosphorus groups in first intermediate to form a second intermediate represented by the following structural formula, or a stereoisomer thereof:



c) optionally capping X^6 groups which did not react with the trivalent phosphorus multimer in step a);

d) treating the second intermediate to remove R^1 to form a 5'- deprotected oligonucleotide; and

e) optionally repeating steps a)-d) one or more times, wherein the final step is step c) or step d), thereby preparing an oligonucleotide.

16. The method of Claim 15, wherein R^{13} is a solid support.

17. The method of Claim 16, further comprising the step of cleaving the oligonucleotide product from the solid support.

18. The method of Claims 15, 16 or 17, further comprising deprotecting the nucleotide bases of the oligonucleotide.

19. The method of Claim 15, wherein X^2 is -O- and R^{13} is an alcohol protecting group.

20. The method of Claim 19, further comprising the step of removing the R^{13} protecting group from the oligonucleotide product.

21. The method of Claims 15, 16, 17, 18, 19 or 20, wherein R^1 is an unsubstituted trityl, a monoalkoxytrityl, a dialkoxytrityl, a trialkoxytrityl, tetrahydropyranyl or a pixyl group.

22. The method of Claim 21, wherein R^1 is removed with an acid selected from a solution of dichloroacetic acid in dichloromethane and a solution of trichloroacetic acid in dichloromethane.

23. The method of any one of Claims 15 to 21, wherein each R^3 is $-OCH_2CH_2CN$ or $-SCH_2CH_2CN$.

24. The method of Claim 23, further comprising removing $-CH_2CH_2CN$ from $-OCH_2CH_2CN$ or $-SCH_2CH_2CN$ by treating the oligonucleotide with a base.

25. The method of Claim 24, wherein the nucleotide bases are deprotected during the treatment of the oligonucleotide with a base.

26. The method of any one of Claims 15 to 25, wherein the trivalent phosphorus groups are oxidized by treating first intermediate with a solution containing I_2 and water.

27. The method of any one of Claims 15 to 25, wherein the trivalent phosphorus groups are sulfurized by treating the first intermediate with 3-amino-[1,2,4]-dithiazole-5-thione or *3H*-benzodithiol-3-one 1,1-dioxide.

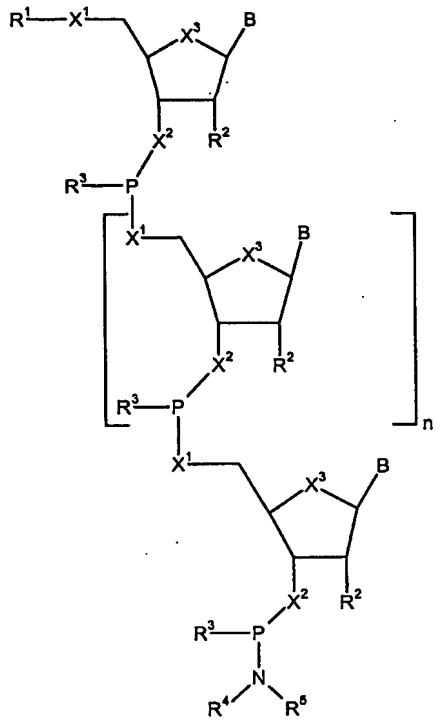
28. The method of any one of Claims 15 to 25, wherein more than one cycle of steps a), b), c) and d) is performed, and the oligonucleotide produced is a chimeric oligonucleotide.

29. The method of any one of Claims 15 to 26, wherein the oligonucleotide produced is a phosphate.

30. The method of any one of Claims 15 to 27, wherein the oligonucleotide produced is a phosphorothioate.

31. The method of any one of Claims 15 to 30, wherein the oligonucleotide prepared has up to 50 nucleotide bases.

32. A method of preparing a trivalent phosphorus multimer represented by the following structural formula, or a stereoisomer thereof:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or -NR-;

each X^3 is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$;

R^1 is a protecting group;

each R^2 is, independently, -H, -F, $-NHR^6$, $-CH_2R^6$ or $-OR^6$;

each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^7$, or $-SR^7$;

R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocycloalkyl group or a heteroaromatic group;

R is -H, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aromatic group, or an amine protecting group;

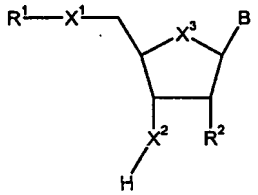
R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or a protecting group;

R^7 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group or a substituted or unsubstituted aralkyl;

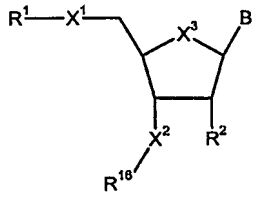
each B is, independently, H or a protected or an unprotected nucleoside base; and

n is 0 or a positive integer, comprising the steps of:

a) protecting the 3'-substituent of a nucleoside represented by the following structural formula, or a stereoisomer thereof:



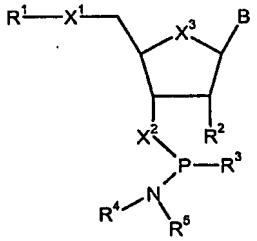
thereby forming a first intermediate represented by the following structural formula, or a stereoisomer thereof:



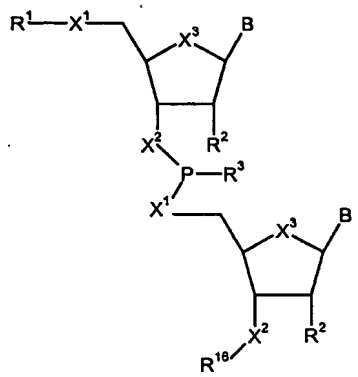
wherein R^{16} is a protecting group which is orthogonal to R^1;

b) treating the first intermediate to remove R^1, thereby forming a 5'-deprotected nucleoside;

c) reacting the 5'-deprotected nucleoside in the presence of a coupling catalyst with a compound represented by the following structural formula, or a stereoisomer thereof:

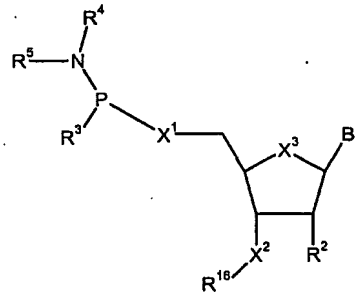


thereby forming a dimer represented by the following structural formula, or a stereoisomer thereof:

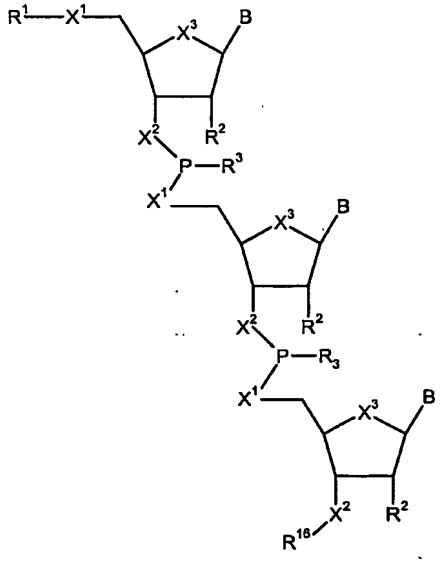


d) treating the 3',5'-protected multimer to remove R^{16}, thereby forming a 3'-deprotected dimer;

e) optionally reacting in the presence of a coupling catalyst the 3'-deprotected dimer with a compound represented by the following structural formula, or a stereoisomer thereof:



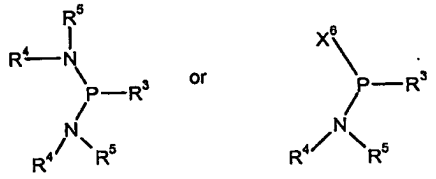
thereby forming a 3',5'-protected trimer represented by the following structural formula, or a stereoisomer thereof:



f) optionally repeating steps e) and f) one or more times, thereby forming a 3',5'-protected multimer;

g) treating the 3',5'-protected multimer to remove R^{16}, thereby forming a 3'-deprotected multimer;

h) reacting the 3'-deprotected multimer with a trivalent phosphorus compound represented by one of the following structural formulas:

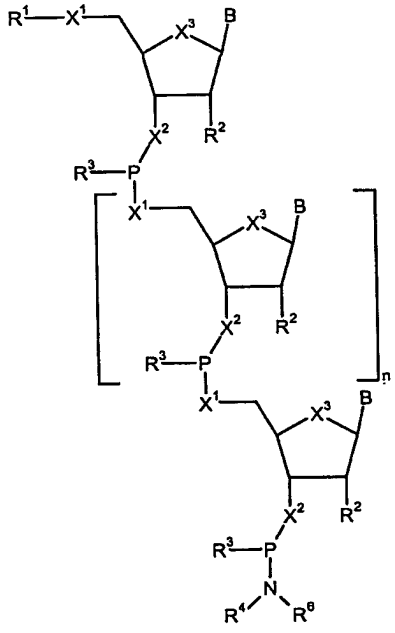


wherein X^6 is a halogen, thereby forming the trivalent phosphorus multimer.

33. The method of Claim 32, wherein the coupling catalyst is tetrazole or S-ethylthiotetrazole.

34. The method of Claims 32 or 33, wherein R^{16} is levulynoyl and is removed by treating the 3',5'-protected multimer with hydrazine hydrate in a pyridine/acetic acid.

35. A method of preparing a trivalent phosphorus multimer represented by the following structural formula, or a stereoisomer thereof:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or -NR-;

each X^3 is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$;

R^1 is a protecting group;

each R^2 is, independently, -H, -F, $-NHR^6$, $-CH_2R^6$ or $-OR^6$;

each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^7$, or $-SR^7$;

R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocycloalkyl group or a heteroaromatic group;

R is -H, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aromatic group, or an amine protecting group;

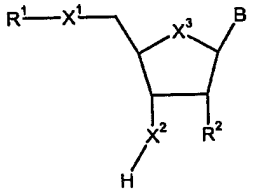
R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or a protecting group;

R^7 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group or a substituted or unsubstituted aralkyl;

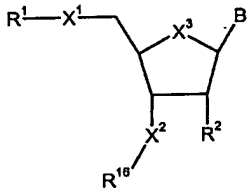
each B is, independently, H or a protected or an unprotected nucleoside base; and

n is 0 or a positive integer, comprising the steps of:

a) protecting the 3'-substituent of a nucleoside represented by the following structural formula, or a stereoisomer thereof:



thereby forming a first intermediate represented by the following structural formula, or a stereoisomer thereof:

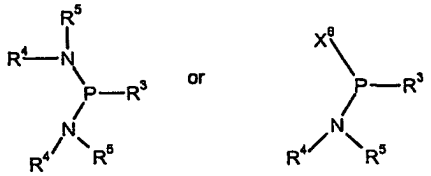


wherein R^{16} is a protecting group which is orthogonal to R^1;

b) treating the first intermediate to remove R^1, thereby forming a 5'-

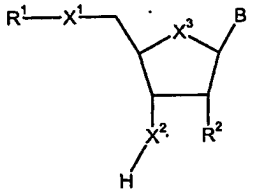
deprotected nucleoside;

c) reacting the 5'-deprotected nucleoside with a trivalent phosphorus compound represented by one of the following structural formulas:

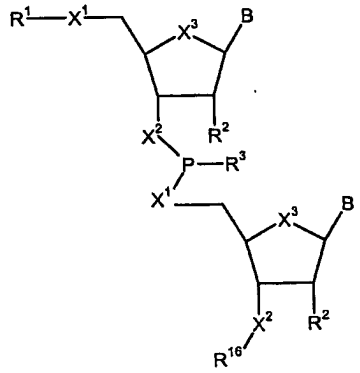


wherein X^6 is a halogen, thereby forming a 5'-phosphoramidite;

d) reacting the 5'-phosphoramidite in the presence of a coupling catalyst with a compound represented by the following structural formula, or a stereoisomer thereof:

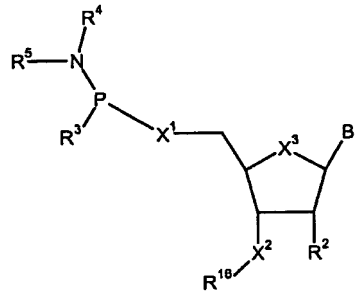


thereby forming a 3',5'-protected dimer represented by the following structural formula, or a stereoisomer thereof:

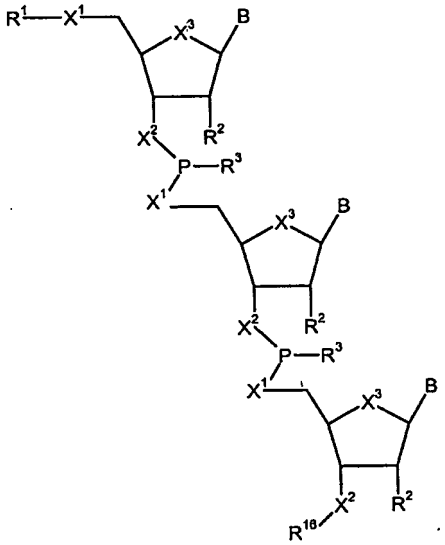


e) treating the 3',5'-protected dimer to remove R^{16}, thereby forming a 3'-deprotected dimer;

f) optionally reacting in the presence of a coupling catalyst the 3'-deprotected dimer with a compound represented by the following structural formula, or a stereoisomer thereof:



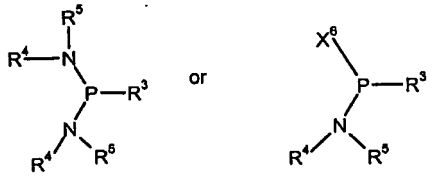
thereby forming a 3',5'-protected trimer represented by the following structural formula, or a stereoisomer thereof:



g) optionally repeating steps e) and f) one or more times, thereby forming a 3',5'-protected multimer;

h) treating the 3',5'-protected multimer to remove R^{16}, thereby forming a 3'-deprotected multimer;

i) reacting the 3'-deprotected multimer with a trivalent phosphorus compound represented by one of the following structural formulas:



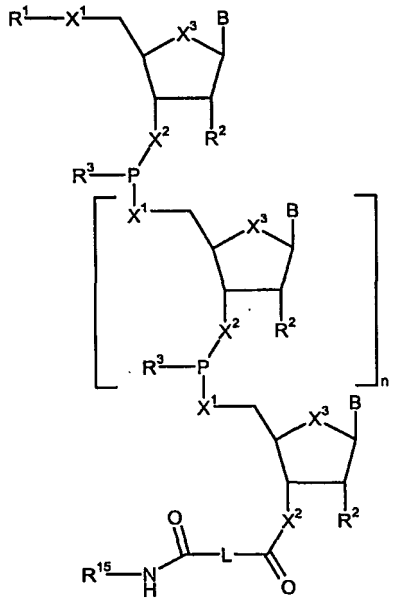
wherein X^6 is a halogen, thereby forming the trivalent phosphorus multimer.

36. The method of Claim 35, wherein the coupling catalyst is tetrazole or S-

ethylthiotetrazole.

37. The method of Claims 30 or 36, wherein R^{16} is levulynoyl and is removed by treating the 3',5'-protected multimer with hydrazine hydrate in a pyridine/acetic acid.

38. A trivalent phosphorus multimer derivatized solid support represented by the following structural formula, or a stereoisomer thereof:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or -NR-;

each X^3 is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$;

R^1 is a protecting group;

each R^2 is, independently, -H, -F, $-NHR^6$, $-CH_2R^6$ or $-OR^6$;

each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^7$, or $-SR^7$;

R is -H, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aromatic group, or an amine protecting group;

R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or a protecting group;

R^7 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group or a substituted or unsubstituted aralkyl;

each B is, independently, H or a protected or an unprotected nucleoside base;

L is a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group;

n is 0 or a positive integer; and

R^{15} is a solid support.

39. The solid support of Claim 38, wherein each X^1, X^2 and X^3 is -O-.

40. The solid support of Claims 38 or 39, wherein R^3 is $-OCH_2CH_2CN$.

41. The solid support of Claims 38. 39 or 40, wherein R^1 is an acid labile protecting group.

42. The solid support of Claim 41, wherein R^1 is 4,4'-dimethoxytrityl.

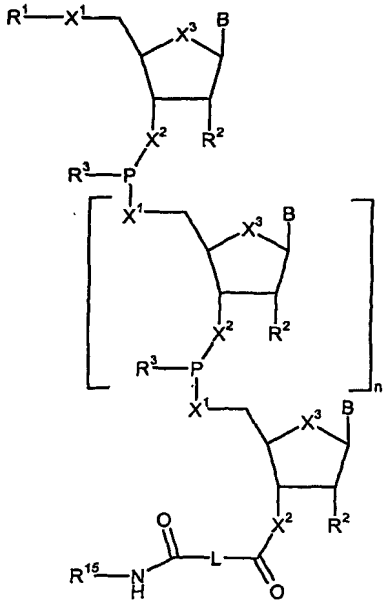
43. The solid support of Claims 38, 39, 40, 41 or 42, wherein R^2 is -H.

44. The solid support of Claims 38, 39, 40, 41 or 42, wherein R_2 is $-OR^6$ and R^6 is a hydroxy protecting group.

45. The solid support of any one of Claims 38 to 44, wherein L is $-CH_2CH_2-$.

46. The solid support of any one of Claims 38 to 45, wherein R^{15} comprises controlled-pore glass, polystyrene or microporous polyamide.

47. A method of preparing a multimer derivatized solid support represented by the following structural formula, or a stereoisomer thereof:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or -NR-;

each X^3 is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$;

R^1 is a protecting group;

each R^2 is, independently, -H, -F, $-NHR^6$, $-CH_2R^6$ or $-OR^6$;

each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^7$, or $-SR^7$;

R is -H, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aromatic group, or an amine protecting group;

R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or a protecting group;

R^7 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group or a substituted or unsubstituted aralkyl;

each B is, independently, H or a protected or an unprotected nucleoside base;

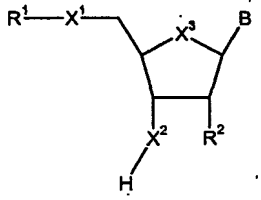
L is a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group;

n is 0 or a positive integer; and

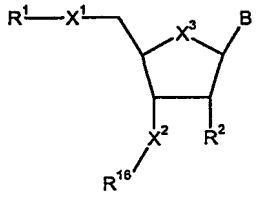
R^{15} is a solid support, comprising the steps of:

a) protecting a 3'-substituent of a nucleoside represented by the following

structural formula, or a stereoisomer thereof:



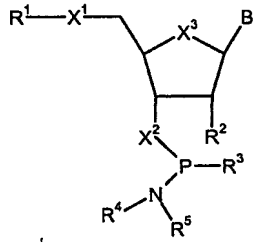
thereby forming a first intermediate represented by the following structural formula, or a stereoisomer thereof:



wherein R^{16} is a protecting group which is orthogonal to R^1;

b) treating the first intermediate to remove R^1, thereby forming a 5'-deprotected nucleoside;

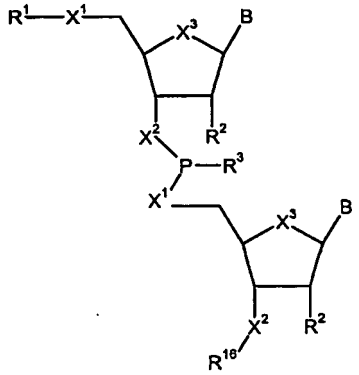
c) reacting the 5'-deprotected nucleoside in the presence of a coupling catalyst with a compound represented by the following structural formula, or a stereoisomer thereof:



wherein:

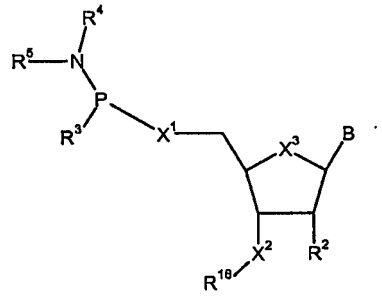
R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocycloalkyl group or a heteroaromatic group; thereby forming a dimer represented by the following structural formula, or a stereoisomer thereof:

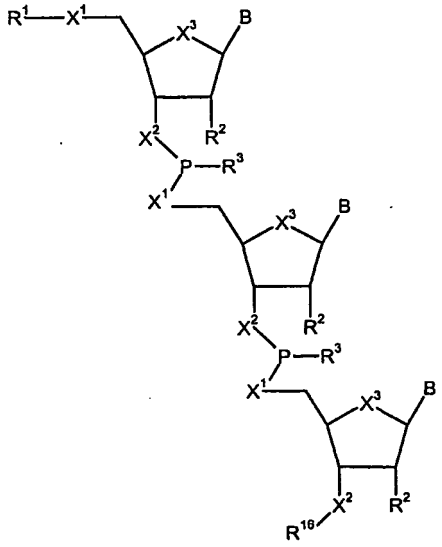


d) treating the 3',5'-protected dimer to remove R^{16}, thereby forming a 3'-deprotected dimer;

e) optionally reacting in the presence of a coupling catalyst the 3'-deprotected dimer with a compound represented by the following structural formula, or a stereoisomer thereof:



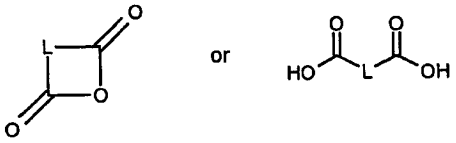
thereby forming a 3',5'-protected trimer represented by the following structural formula, or a stereoisomer thereof:



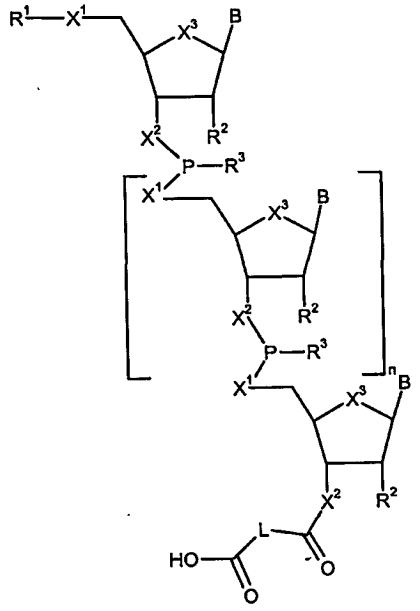
f) optionally repeating steps e) and f) one or more times, thereby forming a 3',5'-protected multimer;

g) treating the 3',5'-protected multimer to remove R^{16}, thereby forming a 3'-deprotected multimer;

h) reacting the 3'-deprotected multimer in the presence of a base with a compound selected from the group consisting of:



thereby forming a solid support loading reagent represented by the following structural formula, or a stereoisomer thereof:



; and

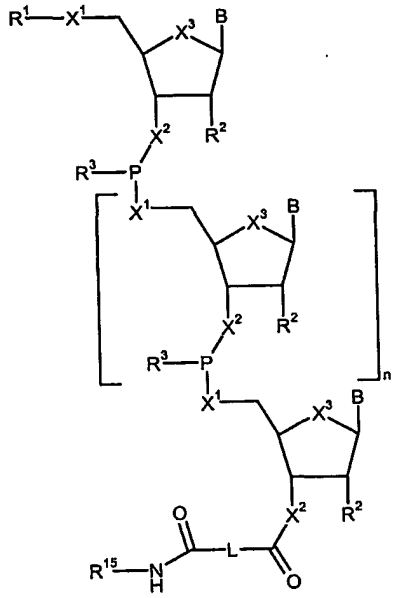
i) reacting the solid support loading reagent with a solid support functionalized with primary or secondary amine groups in the presence of a base and a substituted or unsubstituted dialiphatic carbodiimide, thereby preparing said multimer derivatized solid support.

48. The method of Claim 47, further comprising the step of reacting the solid support loading reagent formed in step f) with p-nitrophenol in the presence of a base and a substituted or unsubstituted dialiphatic carbodiimide, thereby forming an activated solid support loading reagent.

49. The method of Claim 48, wherein the substituted or unsubstituted dialiphatic

carbodiimide is dicyclohexyl carbodiimide or diisopropyl carbodiimide.

50. A method of preparing a multimer derivatized solid support represented by the following structural formula, or a stereoisomer thereof:



wherein:

each X^1 is, independently, -O- or -S-;

each X^2 is, independently, -O-, -S-, or -NR-;

each X^3 is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$;

R^1 is a protecting group;

each R^2 is, independently, -H, -F, $-NHR^6$, $-CH_2R^6$ or $-OR^6$;

each R^3 is, independently, $-OCH_2CH_2CN$, $-SCH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-OR^7$, or $-SR^7$;

R is -H, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aromatic group, or an amine protecting group;

R^6 is -H, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl, or a protecting group;

R^7 is a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group or a substituted or unsubstituted aralkyl;

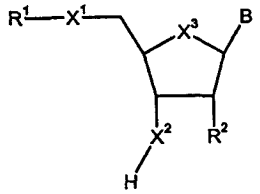
each B is, independently, H or a protected or an unprotected nucleoside base;

L is a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group;

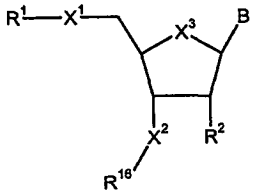
n is 0 or a positive integer; and

R^{15} is a solid support, comprising the steps of:

a) protecting the 3'-substituent of a nucleoside represented by the following structural formula, or a stereoisomer thereof:



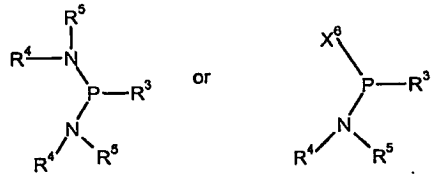
thereby forming a first intermediate represented by the following structural formula, or a stereoisomer thereof:



wherein R^{16} is a protecting group which is orthogonal to R^1;

b) treating first intermediate with an acid to remove R^1, thereby forming a 5'-deprotected nucleoside;

c) reacting the 5'-deprotected nucleoside with a trivalent phosphorus compound represented by one of the following structural formulas:



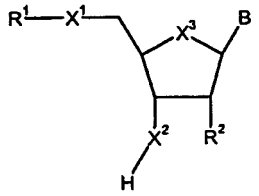
wherein:

X^6 is a halogen, thereby forming a 5'-phosphoramidite, thereby forming a 5'-phosphoramidite; and

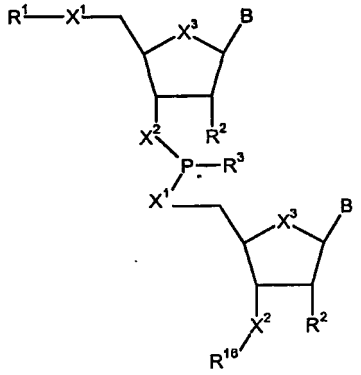
R^4 and R^5 are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic group, a substituted or unsubstituted aralkyl; or

R^4 and R^5 taken together with the nitrogen to which they are bound form a heterocycloalkyl group or a heteroaromatic group;

d) reacting the 5'-phosphoramidite in the presence or a coupling catalyst with a compound represented by the following structural formula, or a stereoisomer thereof:

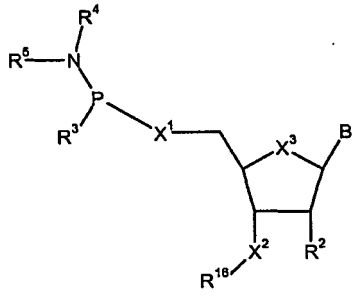


thereby forming a 3',5'-protected dimer represented by the following structural formula, or a stereoisomer thereof:

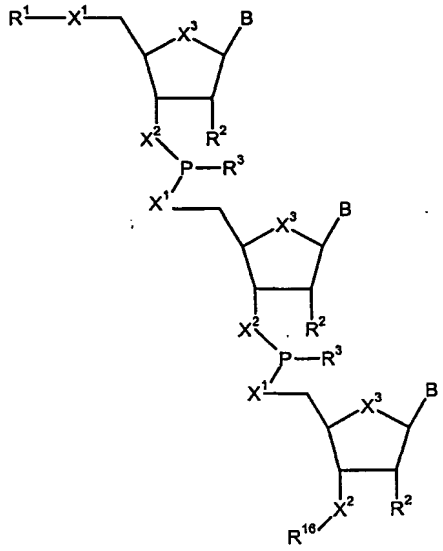


e) treating the 3',5'-protected dimer to remove R_{16}, thereby forming a 3'-deprotected dimer;

f) optionally reacting in the presence of a coupling catalyst the 3'-deprotected dimer with a compound represented by the following structural formula, or a stereoisomer thereof:



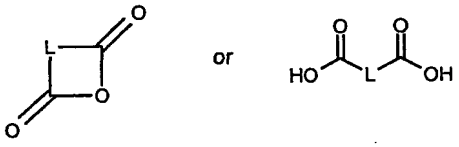
thereby forming a 3',5'-protected trimer represented by the following structural formula, or a stereoisomer thereof:



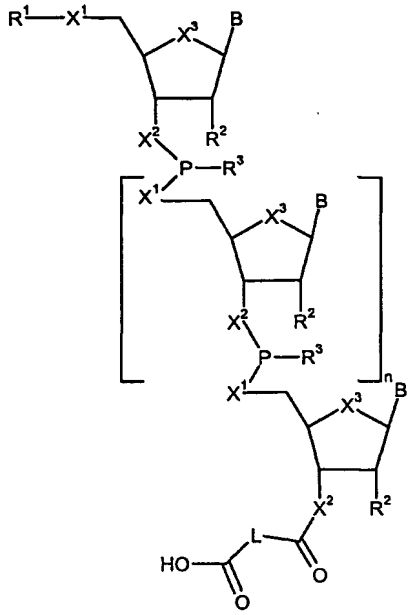
g) optionally repeating steps e) and f) one or more times, thereby forming a 3',5'-protected multimer;

h) treating the 3',5'-protected multimer to remove R^{16}, thereby forming a 3'-deprotected multimer;

i) reacting the 3'-deprotected multimer in the presence of a base with a compound selected from the group consisting of:



thereby forming a solid support loading reagent represented by the following structural formula, or a stereoisomer thereof:



; and

j) reacting the solid support loading reagent with a solid support functionalized with primary or secondary amine functional in the presence of a base and a substituted or unsubstituted dialiphatic carbodiimide, thereby preparing said multimer derivatized solid support.

51. The method of Claim 50, further comprising the step of reacting the solid support loading reagent formed in step h) with p-nitrophenol in the presence of a base and a substituted or unsubstituted dialiphatic carbodiimide, thereby forming an activated solid support loading reagent.

52. The method of Claim 51, wherein the substituted or unsubstituted dialiphatic carbodiimide is dicyclohexyl carbodiimide or diisopropyl carbodiimide.

53. Use of a phosphoramidite compound according to any one of claims 1 to 5 for the synthesis of oligonucleotides.

54. Use of a trivalent phosphorus multimer according to any one of claims 6 to 14 for the synthesis of oligonucleotides.

55. Use of a trivalent phosphorus multimer derivatized solid support according to any one of claims 38 to 46 for the synthesis of oligonucleotides.

FIGURE 1

Figure 1: Method 1 for synthesising a trivalent phosphorus multimer. (DMT = 4,4'-dimethoxytrityl; C^{Bz} = benzoyl protected cytosine, G^{iBu} = isobytyryl protected guanine).

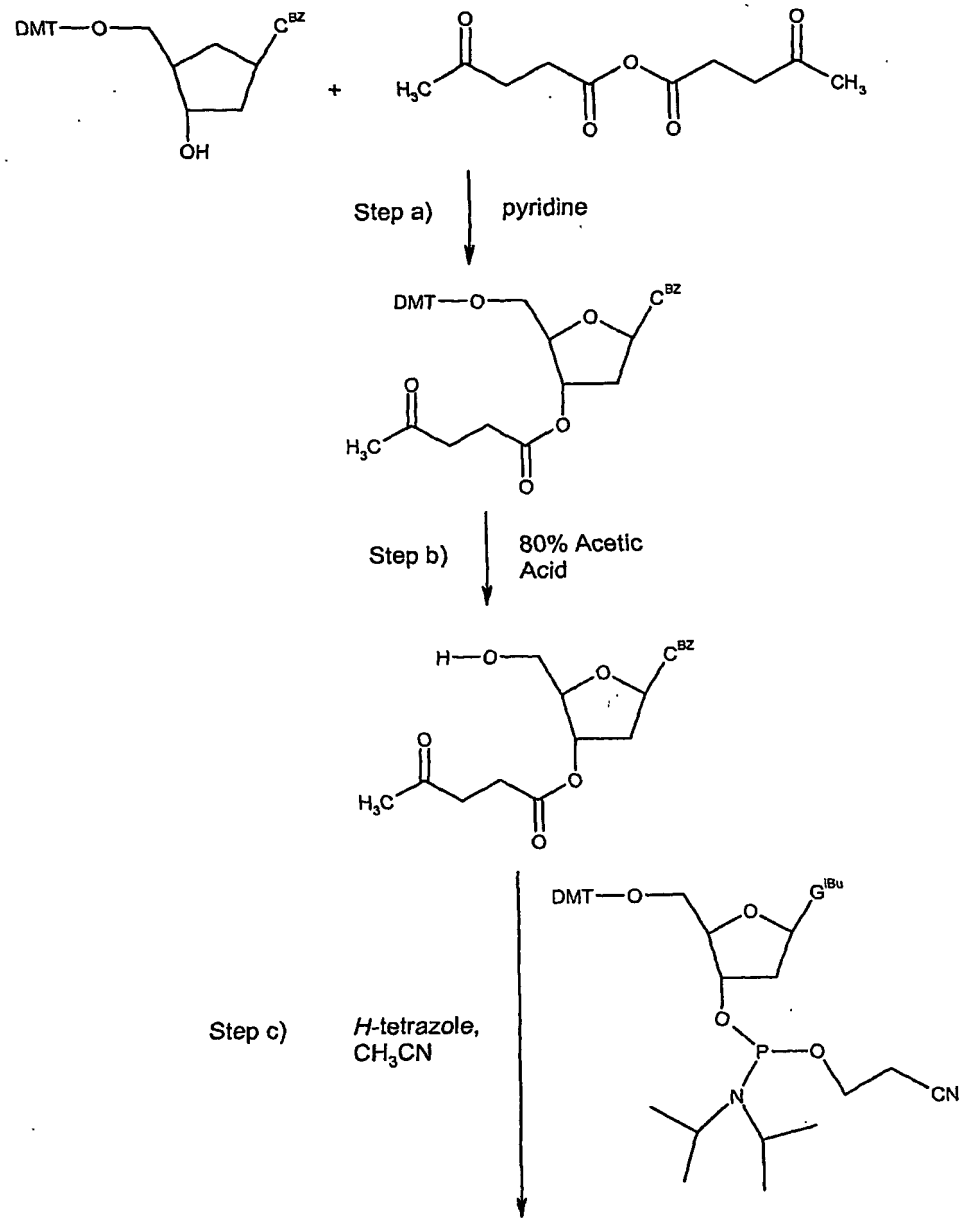


Figure 1 (continued)

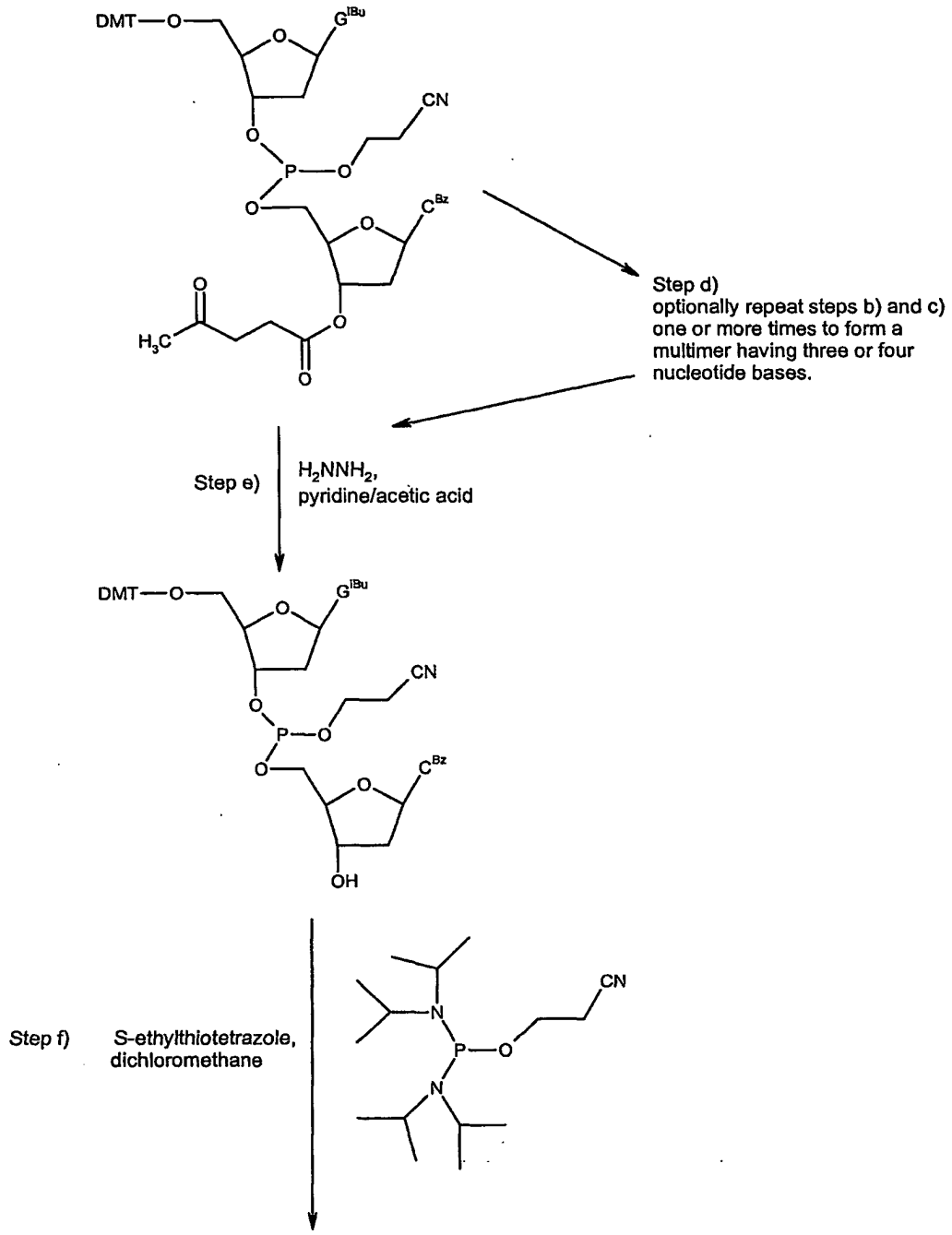
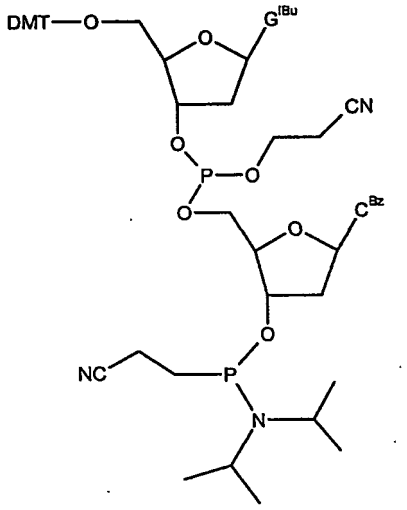


Figure 1 (continued)



Trivalent phosphorous dimer

FIGURE 2

Figure 2: Method 2 for synthesising a trivalent phosphorous multimer. (DMT = 4,4'-dimethoxytrityl; T = thymine, G^{IBu} = isobytyryl protected guanine)

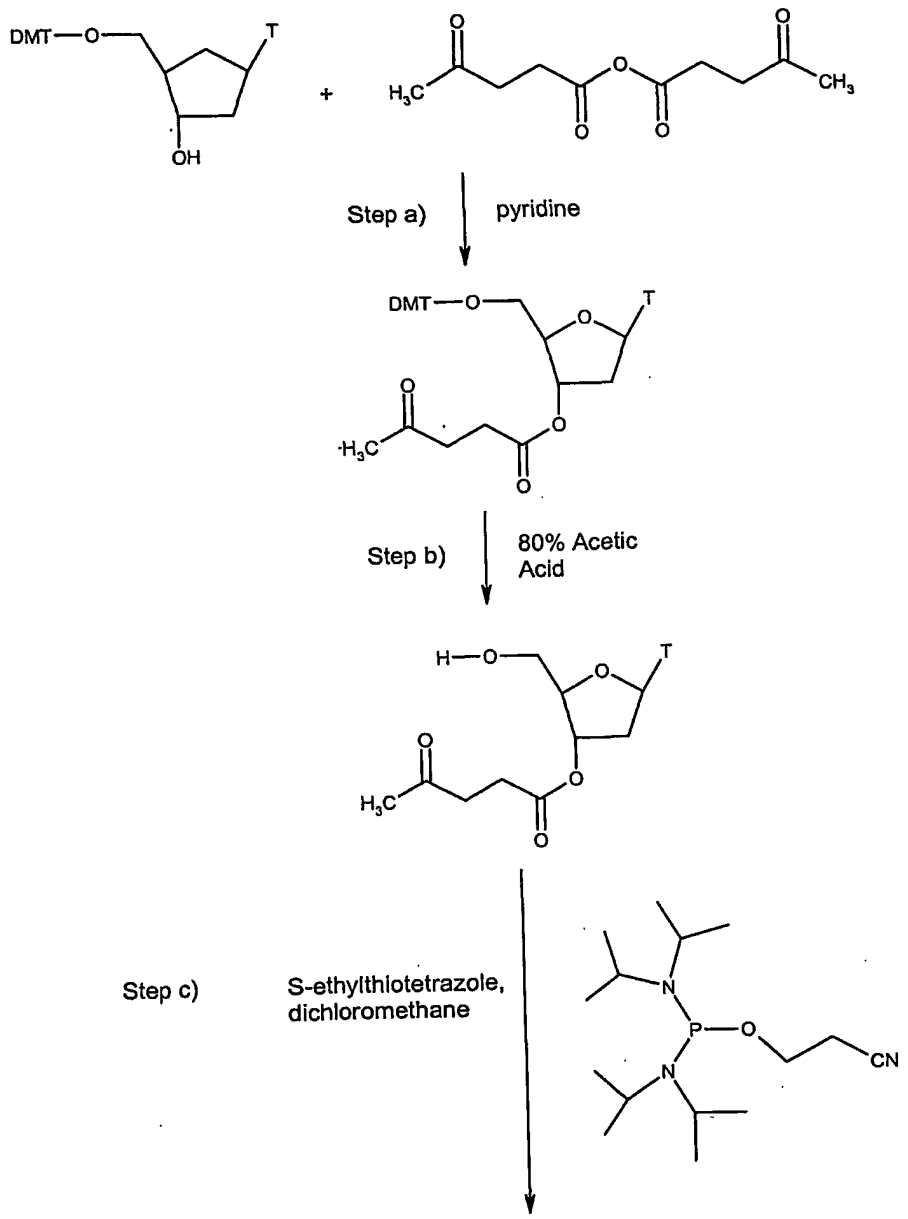


Figure 2 (continued)

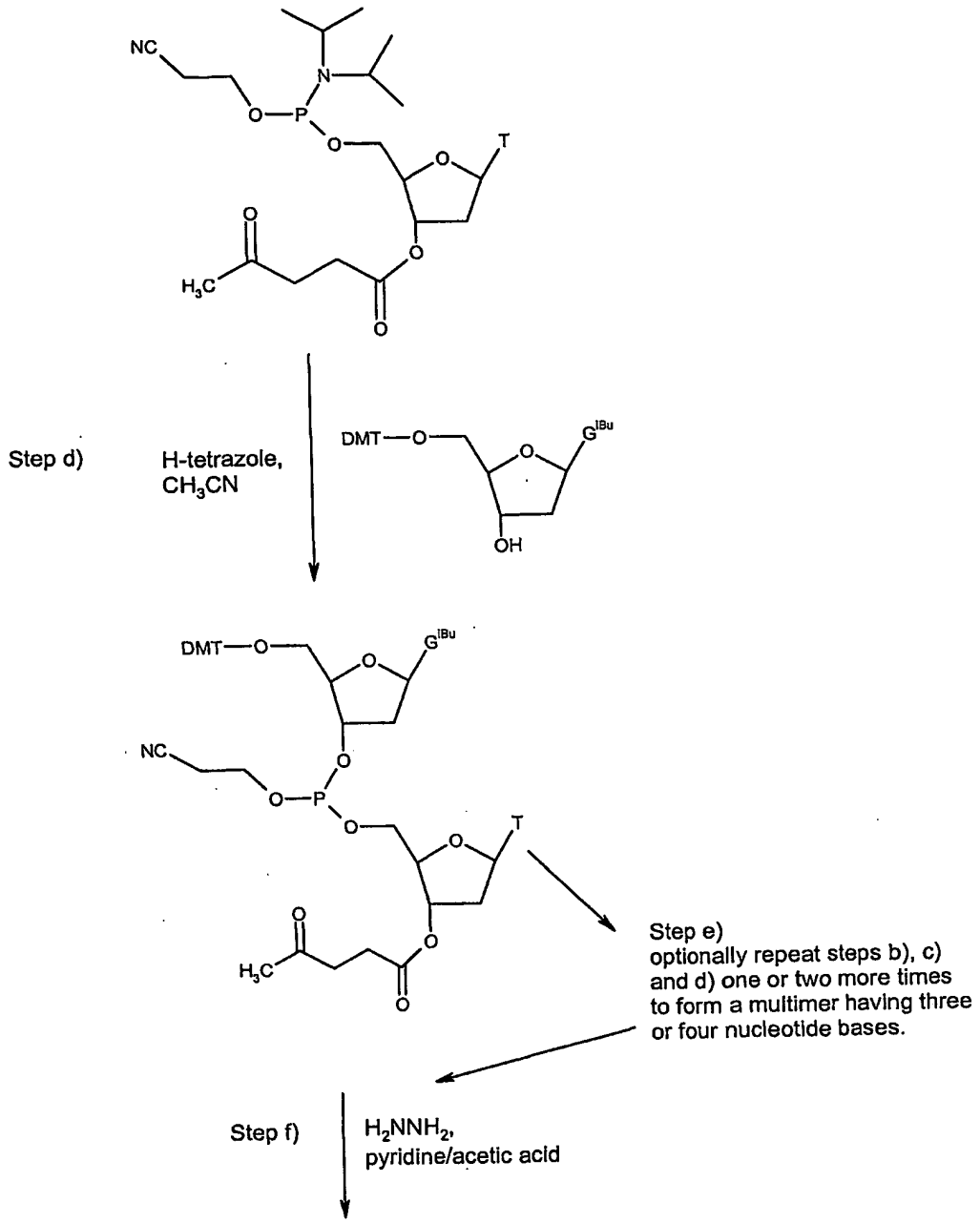


Figure 2 (continued)

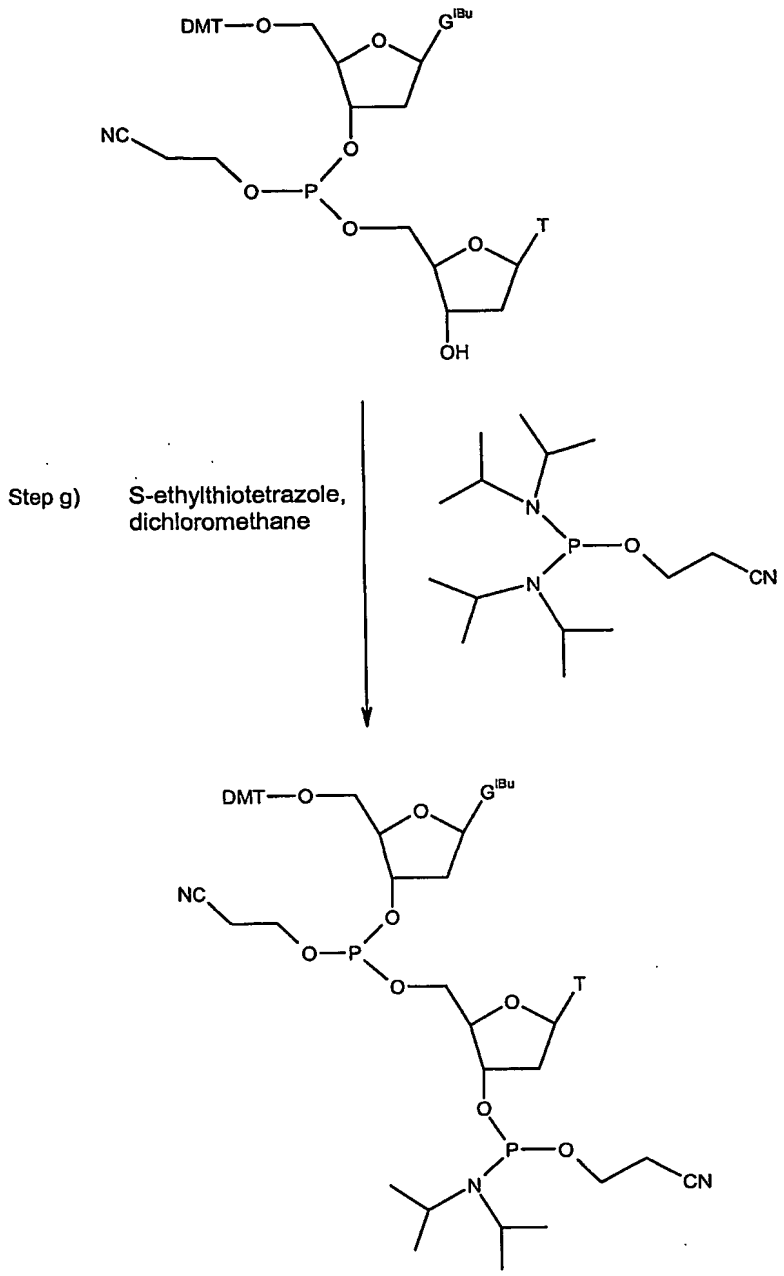


FIGURE 3

Figure 3: 2'-O-Methyl Dimer prepared in Example 2.

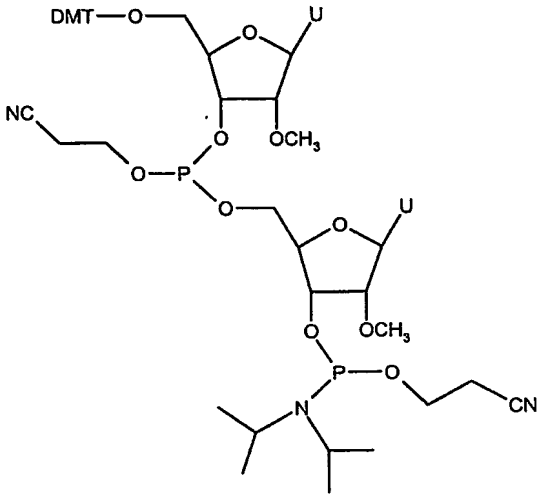
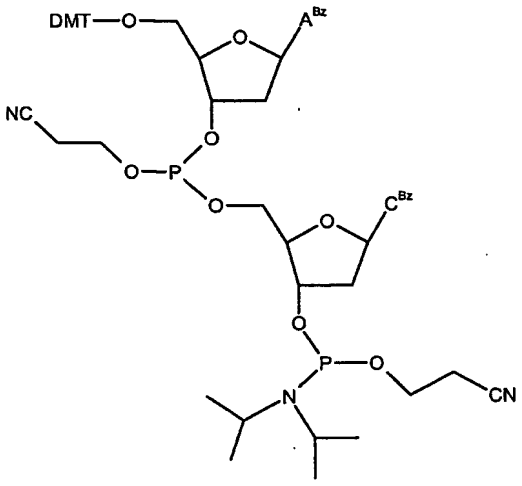
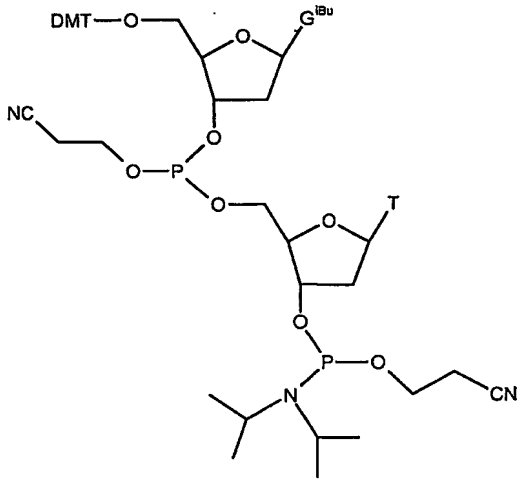


FIGURE 4

Figure 4: Dimers used to synthesize oligonucleotides in Example 4.



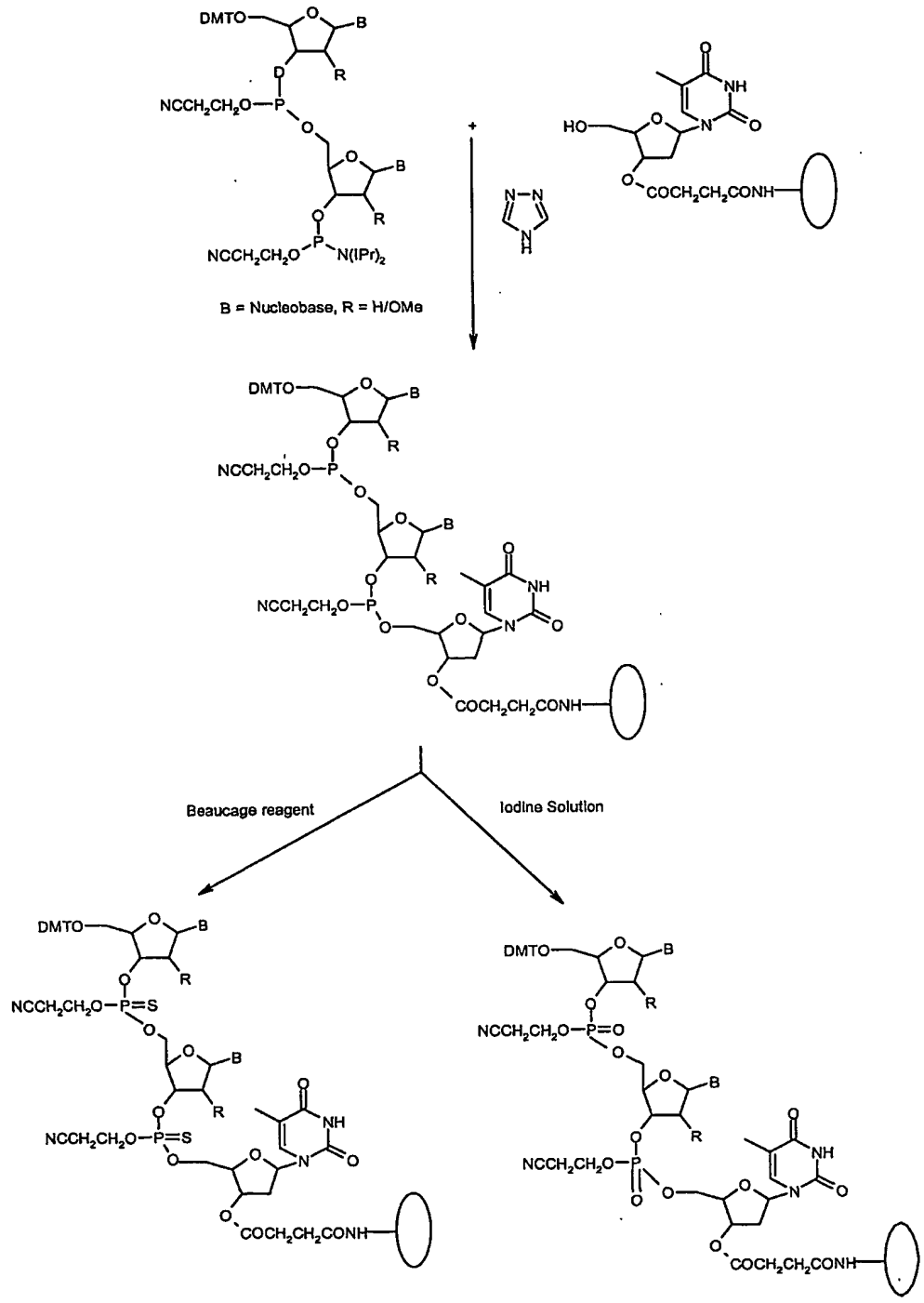
d(AC)



d(GT)

FIGURE 5

Figure 5: Synthesis of oligonucleotides using trivalent phosphorus dimmers.



SEQUENCE LISTING

<110> Avecia Biotechnology, Inc. et al

<120> Synthons for oligonucleotide synthesis

<130> SMC 60437/WO

<160> 2

<170> PatentIn version 3.1

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